

Safeguarding Food:

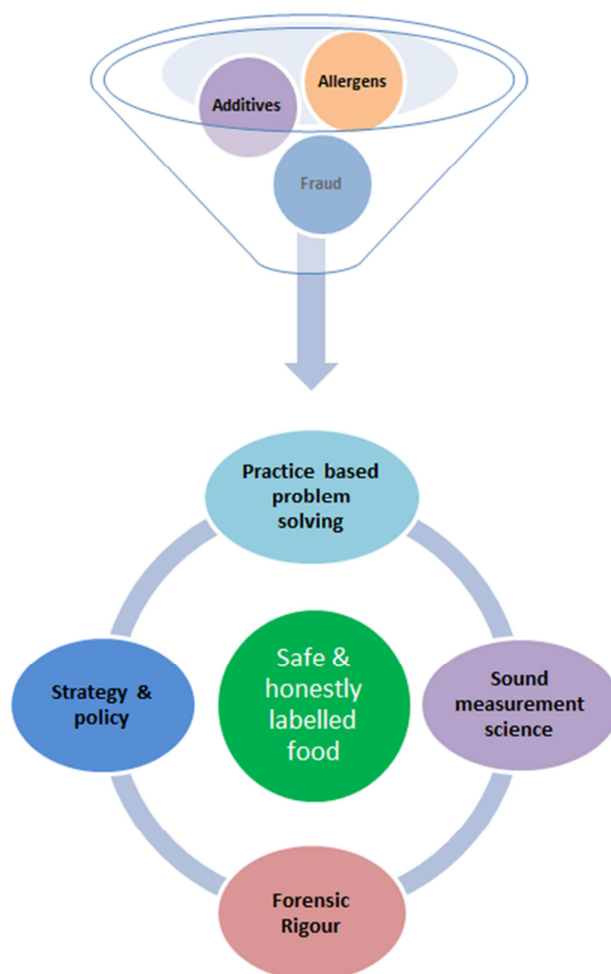
Advances in forensic measurement science and the regulation of allergens, additives and authenticity

Michael John WALKER

Commentary (Volume 1) and Publications (Volume 2) submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy from Kingston University

September 2016

Volume 1: Commentary on publications



Contents

1	Acknowledgements	5
2	Abstract	6
3	Introduction.....	8
3.1	The research outputs	9
3.2	Regulation of Food Safety & Standards.....	13
3.2.1	The 19 th and early 20 th centuries.....	13
3.2.2	Modern regulation of food.....	16
3.3	Career Progression	20
3.4	Research themes overview	22
3.4.1	Food Additives	26
3.4.2	Food Allergens.....	27
3.4.3	Food Authenticity	27
3.4.4	Chemico-legal practice	28
3.5	Summary	29
4	Food Additives - Discussion.....	30
4.1	Permitted Additives	30
4.1.1	Aspartame and PKU	31
4.1.2	Children's behaviour and the 'Southampton' colours.....	32
4.1.3	Baseline additive data.....	32
4.1.4	Non-compliances	33
4.1.5	Outcomes – surveillance reports.....	34
4.1.6	Analysis.....	34
4.2	Non-permitted food additives – gels, morpholine & dimethyl yellow	34
4.2.1	Additives as an acute health risk – choking on jelly mini-cups	35
4.2.2	Morpholine	38
4.2.3	Illegal dyes - dimethyl yellow	40
5	Food Allergens and Food Allergy - Discussion.....	43
5.1	Introduction.....	43
5.1.1	Gambling your life on a take-away meal	43
5.1.2	Food Allergy training.....	45
5.1.3	Coeliac disease and 'gluten-free' food in catering	46
5.1.4	Food Allergen analysis	48
5.1.5	Analysis – mass spectrometry.....	50
5.1.6	Analysis - ELISA ring trial	52
5.2	Forensic implications: Food sabotage – a Crown Court and other cases.....	54

5.2.1	Food Sabotage	54
5.2.2	Food allergy cases in the criminal and civil courts of the UK.....	58
5.3	Food Allergy – review of current knowledge	63
5.3.1	What is Food Allergy?.....	63
5.3.2	Allergen nomenclature.....	66
5.3.3	Allergy Prevalence.....	66
5.3.4	Anaphylaxis.....	71
5.3.5	Severity of allergic reaction	72
5.3.6	Quality of Life	74
5.3.7	Is There a Cure for Food Allergy?	75
5.3.8	Prevention of food allergy	76
5.3.9	Food allergen management.....	78
5.3.10	Processing	80
5.3.11	Precautionary Allergen Labelling.....	81
5.3.12	Basic Toxicology.....	83
5.3.13	Allergen Reference Doses, Action Limits and Thresholds.....	88
5.3.14	Deterministic allergen risk assessment	95
5.3.15	Probabilistic allergen risk assessment	97
6	Food Authenticity – Discussion	101
6.1	Introduction.....	101
6.2	QUID - Quantitative Ingredients Declaration.....	101
6.2.1	Nitrogen factors – a critical review.....	103
6.2.2	Nitrogen factors for farmed salmon and salmon frame mince	105
6.3	Forensic molecular biology	107
6.4	Horse meat 2013.....	109
6.5	Lessons from the past – butter and margarine	112
7	Chemico-legal practice.....	113
7.1	Introduction.....	113
7.2	Food irradiation.....	113
7.3	Review of referee cases.....	114
7.4	Alcohol back calculations	115
7.5	Protection of the Agri-Food Chain by Chemical Analysis: The European Context.....	116
7.6	Achieving Quality Chemical Measurements in Foods	116
8	Conclusions	118
8.1	Introduction.....	118
8.2	Food Surveillance.....	119
8.3	Food Authenticity – the Elliott Review.....	121
8.4	Food Allergens	124

8.4.1	Almond or Peanut?	124
8.4.2	Almond or mahaleb?.....	125
8.4.3	Allergen analysis.....	126
8.4.4	A framework to address the problems.....	128
8.4.5	Reference materials.....	128
8.4.6	Metrologically traceable methods	132
8.4.7	International collaboration	133
9	References	136
10	Citations of Walker's work	153
11	Appendix 1 Author Contributions	162

1 Acknowledgements

It is a pleasure to acknowledge the support of my wife Maria and daughters Julia (and family) and Elaine and James whose benign acceptance of my lengthy preoccupation has resulted in the publications herein. It is impossible to do credit in a short acknowledgement to the extent of Maria's positive encouragement of my science career. Maria's practical help as we have worked together throughout my professional career has been invaluable.

I also acknowledge with gratitude Professor Duncan Thorburn Burns FRSE MRIA from whom I have learned much, not least about evaluation of evidence and construction of a paper for the peer reviewed literature. Professor Burns' tuition, encouragement and help throughout my career is characteristic of his dedication to his students and his sense of the fun of learning and the practice of analytical science.

I am very grateful to Professor Declan Naughton under whose guidance and encouragement this thesis was assembled and who steered its course through the University of Kingston. Professor Naughton has taken great pains to develop in me the essential skills needed to draft Volume 1 of this portfolio.

I am grateful to the Government Chemist Dr Derek Craston for encouragement and support and to BIS (Business, Innovation & Skills, replaced by Department for Business, Energy & Industrial Strategy in July 2016) for funding for much of the work carried out from 2006 onward.

I am grateful to Environmental Health colleagues for sampling for work carried out prior to 2006 and to Environmental Health Departments in Northern Ireland for funding for the analytical work on food additives and peanut protein in take-away meals.

I am grateful to the Defra, for funding for the Survey of the Up-skilling of the UK Official Food Control System in DNA Food Authenticity Techniques.

Lastly I am grateful to my collaborators for their dedication, hard work, good science and friendly cooperation.

2 Abstract

This commentary reports on work published between 2005 and 2015 forming a record of a varied career building technical competence alongside strategic skills in the analytical chemistry and molecular biology of food. The unifying theme is practice based problem solving in the scientific regulation and enforcement of food safety and authenticity. The work demonstrates advances in sound, forensically robust, measurement science addressing problems arising from food additives, food authenticity and food allergens. In particular the mature discipline that underpins the regulation and enforcement of food additives is shown to be needed for the management of food allergens. The background to food regulation and enforcement is described alongside technical appeals in the official food control system to develop societally meaningful food surveillance, supported by a sustainable UK based official food control infrastructure (Public Analyst service) at the interface between science and the law.

For food additives, publication of previously un-collated results informs regulatory practice and demonstrates the value of scientific collaboration between both jurisdictions on the island of Ireland. A definitive strategy is reported for the chemical analysis and risk assessment of 'jelly mini-cups' in which gel forming additives have caused choking fatalities and solutions to problems in the analysis of two illegal toxic additives, morpholine and dimethyl yellow are described.

For food allergens the portfolio includes the first study to assess in quantitative terms the level of risk to peanut allergic consumers in take-away catering, leading to better training and similar work on coeliac disease and the availability of 'gluten-free' food. Systematic assessment of food allergen analysis and a programme of analytical improvement to support allergen risk assessment and risk management are discussed. A narrative account of an allergen related food sabotage incident and the subsequent Crown Court case and previously uncollated reports of court-sanctions for allergen non-compliances, severe incidents and deaths make key policy and practice recommendations for improvement in these areas.

In the study of food authenticity a critical review describes the nitrogen content of important species in the food supply chain as a proxy in the quantitative estimation of high value flesh foods in compound products. An exemplar study follows determining previously unavailable nitrogen factor data for farmed salmon and salmon frame mince. A critical survey of the upskilling of the UK Official Food Control System in DNA food authenticity techniques and major historical and contemporary reviews of food fraud (butter and horsemeat) support substantial policy and analytical recommendations.

Many threats to our food supply can be assessed and managed only with the assistance of measurement science. Integrating elements of chemico-legal practice including lessons learned from 'referee analyses' and metrology in chemistry this commentary concludes with a synthesis describing major changes in the UK scientific food control system stemming from the author's involvement in the 'Elliott Review' and recommendations for an international programme of work on food allergen analysis with interconnected learning for the benefit of the analytical and regulatory communities and society at large.

3 Introduction

This commentary introduces a body of published work about the analytical chemistry and molecular biology of food. The unifying theme is practice based problem solving in the scientific regulation and enforcement of food safety and authenticity. The research outputs reflect a career in which I have enjoyed building technical competence alongside strategic skills.

Technical skills were developed as Public Analyst (1986 - 2004) and as Referee Analyst in the Laboratory of the Government Chemist (2007 – date). In the former post I was responsible for scientific enforcement of food safety and authenticity in Northern Ireland and in the latter resolve technical appeals in the UK food control system. In parallel strategic and policy skills stem from executive and non-executive Director experience, including as a founder Board member of the Food Standards Agency, (2000 – 2004) and Chief Executive of Forensic Science Northern Ireland (2004 – 2006). A chemico-legal consultancy (2006 to date) better facilitated the development of research and dissemination skills.

The introductory section of Volume 1 describes:

- The background to food regulation and enforcement;
- Technical appeals in the official food control system; and
- A brief review of the research themes, challenges, experience and skills gained, and outcomes arising during my career.

The following sections of Volume 1 describe work in four broad categories:

- Food Additives
- Food Allergens
- Food Authenticity
- Chemico-legal practice

In the final section of Volume 1 conclusions are drawn.

Volume 2 is the collection of published work gathered for the submitted portfolio.

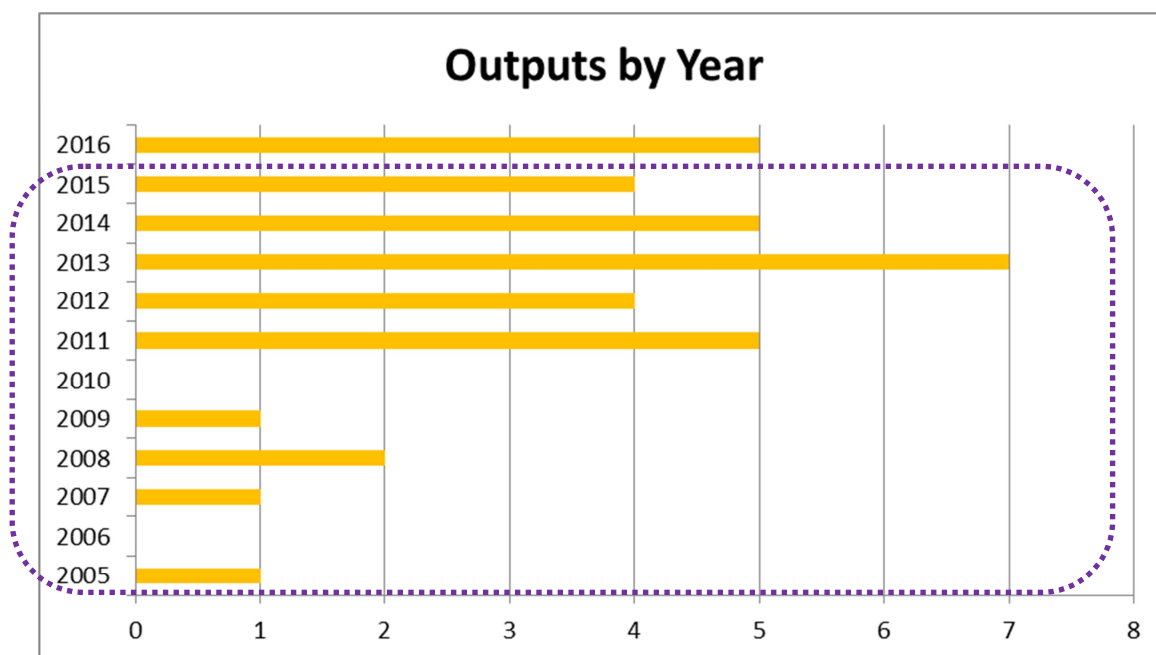


Figure 1 Walker's outputs by year,
(Publications from 2005 to 2015 are included in this portfolio)

3.1 The research outputs

The 26 research outputs in this portfolio are all in the public domain, the majority (24/26) as peer reviewed papers in the scientific literature. The topic category, output type and author contributions are shown in Table 1 below with Walker's contribution classified as follows:

A: Michael Walker made substantial contributions to drafting the output and, depending on the study, to one or more of the following - the conception and design of the study, the organisation of the conduct of the study, carrying out the study (including for some papers acquisition of study data), or analysis and interpretation of study data.

B: Michael Walker helped draft the output; or critique the output for important intellectual content.

Citations of Walker's work are collected in section 10 and in section 11, Appendix 1, 'Author Contributions' contains fuller granularity, attested for each multi-author output by a co-author.

Table 1: Topic category, output, type and contribution

Food Additives

OUTPUT	Paper Type and MJW contribution
[1] A Cross-border Method Validation for the Determination of Sweeteners in Soft Drinks, M. J. Walker , J. McShane, D. Thorburn Burns, P. Canavan and A. Flanagan, <i>Journal of the Association of Public Analysts</i> (Online), 2009, <u>37</u> , 1-14.	Peer reviewed research paper A
[2] Intense Sweeteners and Preservatives: Contemporary Regulation and Historical Baseline Data of the Nature and Amounts in Soft Drinks on open sale in Northern Ireland, M. J. Walker , J. Mairs and D. Thorburn Burns, <i>Journal of the Association of Public Analysts</i> (Online), 2011, <u>39</u> , 1-12.	Peer reviewed research paper A
[3] Analytical Strategy for the Evaluation of a Specific Food Choking Risk, a Case Study on Jelly Mini-Cups, M. J. Walker , P. Colwell, D. Craston, I. P. Axford and J. Crane, <i>Food Analytical Methods</i> , 2012, <u>5</u> (1), 54-61.	Peer reviewed research paper A
[4] Forensically Robust Detection of the Presence of Morpholine in Apples—Proof of Principle M. J. Walker , K. Gray, C. Hopley, D. Bell, P. Colwell, P. Maynard and D. Thorburn Burns, <i>Food Analytical Methods</i> , 2012, <u>5</u> (4), 874–880.	Peer reviewed research paper A
[5] Forensically Robust Determination of the Illegal Dye Dimethyl Yellow in a Refractory Curcuma Oleoresin–Surfactant Matrix—a Case Study, M. J. Walker , P. Colwell, S. Biesenbruch, B. Stuart and D. Thorburn Burns, <i>Food Analytical Methods</i> , 2013, <u>6</u> (2), 395–405.	Peer reviewed research paper A

Food Allergens

OUTPUT	Paper Type and MJW contribution
[6] Food Allergy: Gambling your life on a take-away meal, I. Leitch, M. J. Walker , and R. Davey, <i>International Journal of Environmental Health Research</i> , 2005, <u>15</u> (2), 79-87.	Peer reviewed research paper A
[7] Food allergy training evaluation, 2007, M. J. Walker : http://www.cawt.com/Site/11/Documents/Publications/Population%20Health/safeFoodEvaluation.pdf .	Report to a client A
[8] Food Allergen Detection: A Literature Review 2004 – 2007, M. J. Walker , P. Colwell, S. Elahi, K. Gray and I. Lumley, <i>Journal of the Association of Public Analysts</i> (Online), 2008, <u>36</u> , 1-18.	Peer reviewed critical literature review A
[9] Awareness of coeliac disease and the gluten status of ‘gluten-free’ food obtained on request in catering outlets in Ireland, J. McIntosh, A. Flanagan, N. Madden, M. Mulcahy, L. Dargan, M. J. Walker & D. Thorburn Burns, <i>International Journal of Food Science & Technology</i> , 2011, <u>46</u> , 1569-1574.	Peer reviewed research paper A
[10] Food allergy, a summary of recent cases in the criminal and civil courts of the UK, Oral presentation, H. M. Gowland and M. J. Walker , 2011, <i>Clinical and Translational Allergy</i> , Volume 1, Supplement 1 (2011), O1.	Abstract of oral presentation A
[11] A Mass Spectrometry-based Reference Method for the Analysis of Lysozyme in Wine and the Production of Certified Reference Materials, A. Cryar, C. Pritchard, W. Burkitt, M. J. Walker , G. O'Connor and M. Quaglia, <i>Journal of the Association of Public Analysts</i> (Online), 2012, <u>40</u> , 77-80.	Peer reviewed research paper A

[12] Forensic investigation of a sabotage incident in a factory manufacturing nut-free ready meals in the UK, M. J. Walker , In: J Hoorfar, Ed. Case Studies in food safety and authenticity, Woodhead Publishing, 2012, pp 288-295.	Book chapter A
[13] Towards Absolute Quantification of Allergenic Proteins in Food—Lysozyme in Wine as a Model System for Metrologically Traceable Mass Spectrometric Methods and Certified Reference Materials, A. Cryar, C. Pritchard, W. Burkitt, M. J. Walker , G. O'Connor, D. Thorburn Burns and M. Quaglia, <i>Journal of the Association of Analytical Communities International</i> , 2013, <u>96</u> , 1350-1361	Peer reviewed research paper A
[14] A multi-laboratory evaluation of a clinically-validated incurred quality control material for analysis of allergens in food, P. E. Johnson, N. M. Rigby, J. R. Dainty, A. R. Mackie, U. U. Immer, A. Rogers, P. Titchener, M. Shoji, A. Ryan, L. Mata, H. Brown, T. Holzhauser, V. Dumont, J. A. Wykes, M. J. Walker , J. Griffin, J. White, G. Taylor G., B. Popping, R. Crevel, S. Miguel, P. Lutter, F. Gaskin, T. B. Koerner, D. Clarke, R. Sherlock, A. Flanagan, C.H. Chan, E.N. Clare Mills, <i>Food Chemistry</i> , 2014, <u>148</u> , 30-36.	Peer reviewed research paper B
[15] Food Allergy, a summary of 8 cases in the UK criminal and civil courts: effective last resort for vulnerable consumers?, M. H. Gowland and M.J. Walker , 2014, <i>Journal of the Science of Food and Agriculture</i> , 2015, <u>95</u> (10), 1979-1990.	Peer reviewed desk top critical review A

Food Authenticity

OUTPUT	Paper Type and MJW contribution
[16] Nitrogen factors as a proxy for the quantitative estimation of high value flesh foods in compound products, a review and recommendations for future work, D. Thorburn Burns, M. J. Walker , S. Elahi and P. Colwell, <i>Analytical Methods</i> , 2011, <u>3</u> , 1929-1935.	Peer reviewed desk top critical review A
[17] Nitrogen Factors for Atlantic Salmon, <i>Salmo salar</i> , farmed in Scotland and in Norway and for the derived ingredient, "Salmon Frame Mince", in Fish Products. P. Colwell, S. L. R. Ellison, M. J. Walker , S. Elahi, D. Thorburn Burns and K. Gray, <i>Journal of the Association of Public Analysts</i> (Online), 2011, <u>39</u> , 44-78.	Peer reviewed research paper A
[18] Can Analytical Chemists do Molecular Biology? A Survey of the Up skilling of the UK Official Food Control System in DNA Food Authenticity Techniques, M. Woolfe, T. Gurung and M. J. Walker , <i>Journal of Food Control</i> , 2013, <u>33</u> , 385-392.	Peer reviewed survey research paper A
[19] A Survey of the Up skilling of the UK Official Food Control System in DNA Food Authenticity Techniques, M. J. Walker and M. Woolfe, 2011 (Full Report available at Defra website ¹).	Survey research Report to Defra A
[20] Horse meat in beef products, species substitution, M. J. Walker , M. Burns and D. Thorburn Burns, <i>Journal of the Association of Public Analysts</i> (Online), 2013, <u>41</u> , 67-106.	Peer reviewed research paper A
[21] The adulteration of food, lessons from the past, with reference to butter, margarine and fraud, European Food Research and Technology, H. Deelstra, D. Thorburn Burns and M. J. Walker , <i>European Food Research and Technology</i> , 2014, <u>239</u> , 725-744.	Peer reviewed desk top critical review A

¹

<http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=17627&FromSearch=Y&Publisher=1&SearchText=FA0102&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description>). (Last accessed 26.07.2016)

Chemico-legal practice

OUTPUT	Paper Type and MJW contribution
[22] Referee analysis of suspected irradiated food, S. Elahi, I. Straub, K. Thurlow, P. Farnell, and M. J. Walker , <i>Journal of Food Control</i> , 2008, <u>19</u> , 269-277.	Peer reviewed research paper B
[23] <i>Quis custodiet</i> – a Review of the Resolution of Disputed Chemical Results in the UK Official Feed and Food Control System 2010 – 2011, M. J. Walker and K. Gray, <i>Journal of the Association of Public Analysts</i> (Online) 2013, 41, 1–27.	Peer reviewed research paper A
[24] Alcohol Back Calculations – How far have we come since <i>King - v - Carson?</i> , M. J. Walker and D. Thorburn Burns, Parts 1 & 2, <i>The Writ</i> , the Journal of the Law Society of Northern Ireland, Issue 217, May-June 2013, 14 – 16 and Issue 218, Summer/Autumn 2013, 10–11.	Desk top critical review, not peer reviewed A
[25] Protection of the Agri-Food Chain by Chemical Analysis: The European Context, M. J. Walker and Y. C. Wong, In: Rajeev Bhat, Vicente M. Gomez-Lopez (Eds) <i>Practical Food Safety: Contemporary Issues and Future Directions</i> , May 2014, Wiley-Blackwell, ISBN: 978-1-118-47460-0, 125-144.	Book Chapter A
[26] Achieving Quality Chemical Measurements in Foods, Y. C. Wong and M. J. Walker , In: Rajeev Bhat, Vicente M. Gomez-Lopez (Eds) <i>Practical Food Safety: Contemporary Issues and Future Directions</i> , May 2014, Wiley-Blackwell, ISBN: 978-1-118-47460-0, 99-124.	Book Chapter B

Literature is cited herein in Harvard style, and listed in section 9, however, for clarity Walker's outputs are also identified by the number assigned in Table 1.

Numerically referenced footnotes give additional information.

Technical analytical method details are to be found in the published outputs in Volume 2.

To assist the reader an outline of the regulation and enforcement of food safety and authenticity follows, to place my career progression in context.

3.2 Regulation of Food Safety & Standards

3.2.1 The 19th and early 20th centuries

It is now generally accepted that the responsibility for safe and honestly described food lies with those who produce and sell it. Nevertheless there has been an expectation from society of governmental oversight of food. Food adulteration, evident from the earliest times², increased dramatically with the industrial revolution, Burnett (1958) cited with approval by Hobsbawm (1963). Deelstra, Burns and Walker [21] trace the development of food regulation from the Middle Ages to the early 19th century; from national Guilds, groups of traders throughout Europe, regulating commerce in high-value food (e.g. spices) to the adoption of food control by the State or by local or municipal authorities. Foods such as bread, tea, coffee, beer and wine came to be dealt with by specific statutes, to safeguard the revenue and to protect health. The quality, contamination and adulteration of food was investigated by chemists and medical practitioners in the 19th century, notably by Accum (1820) and Hill Hassall (1855)³ in the UK with parallel developments in Europe and the United States.

French and Phillips (2000) have outlined a series of theoretical models derived from American studies (see for example Law, 2003) of the sources of regulatory policies and argue for the influence of social reformers in introducing food regulation in the public interest. French and Phillips describe the efforts of sections of the food industry to further their own interests in the development of food regulation.

By contrast Deelstra, Burns & Walker [21] emphasise that the scientific investigation of food adulteration and publication of the findings led in the UK to several Select Parliamentary Committees and Acts of Parliament in 1860 and 1872 to counteract food adulteration. These Acts were of limited success, because of, for example, the lack of a definition of “adulteration”,

² “Then by lowering the bushel, raising the shekel, by swindling and tampering with the scales we can buy up the poor for money, and the needy for a pair of sandals, and get a price even for the sweepings of the wheat” Amos, prophet, ~750BC, 8, 5 - 6.

³ Charnley 2005 and 2008 offers a critique of conventional histories of 19th anti-adulteration campaigns, arguing, for example, that the identification of Accum and Hassall as central figures is a selective move; numerous other actors including Wakley, Postgate and Letheby were also crucially involved.

(Wynter Blyth & Wynter Blyth 1909, Clare & Clare 2012). However the Sale of Food and Drugs Act 1875 is widely regarded as a turning point in the regulation of food, introducing key concepts such as that food must be of the 'nature', or 'substance' or 'quality' demanded by the purchaser. It included as a duty of local government the appointment of a certain type of scientist, the Public Analysts, to provide the underpinning analytical data and its interpretation for the enforcement of the provisions of the Act, (Dunlap 1911, Taylor 2010).

Public Analysts in the 19th century were prominent in developing the (analytical) chemistry of food, organic substances generally and water, and contributed to Public Health and medico-legal investigations. Their text books and publications attest to this: see for example Hill Hassall (1855) on food adulteration, Sutton⁴ (1863) on volumetric analysis, Wynter Blyth⁵ (1876 *et post*, cited in Burns 2007) on food analysis, Public Health and toxicology, and Allen's⁶ Commercial Organic Analysis, first published in 1879 and running eventually to a nine volume set (see Clare & Clare 2012 for details of this and Allen's >150 other publications). The Public Analysts founded the *Analyst* and contributed substantially in the 19th century to professional bodies such as the Chemical Society and the Institute of Chemistry of Great Britain and Ireland.

Despite this Oddy (2007)⁷ describes how Public Analysts:

"began work in a climate they perceived as hostile to their very existence. Poor remuneration for analysing and certifying samples of food was the basis for their sense of insecurity but analysts felt themselves to be distrusted even by the local authorities who employed them."

We will return to the situation of the modern Public Analyst service in the concluding section of this volume.

⁴ Francis Sutton (1831 – 1917) Public Analyst for Norfolk (Obituary, A. E. Johnson, 1917, *Analyst*, 42, 261-263.

⁵ Alexander Wynter Blyth (1844-1921) Public Analyst for Devon

⁶ Alfred Henry Allen, (1846 – 1904) Public Analyst for Sheffield

⁷ In a chapter in 'Food and the City in Europe since 1800', published in 2007 and dedicated to the memory of John Burnett cited above. See also <http://www.theguardian.com/news/2006/nov/29/guardianobituaries.mainsection> (accessed 16.03.2016)

Food science and analytical chemistry were in their infancy in the 19th century and many early Public Analyst appointments were held by medical practitioners rather than trained analysts. It is not surprising that there was dissatisfaction expressed with the poor quality of some analyses from inexperienced analysts. Thus the 1875 Act appointed the 'Chemical Officers of Somerset House' as 'analytical referees'. This function evolved into the modern Government Chemist Programme, with a designated Referee Analyst, a post currently held by the writer, but was initially resented by the Public Analysts and for some years there was friction (Hammond and Egan 1992) in part fuelled by lack of publication by the then Government Chemist of his methods and capability. However by the early years of the 20th century cordial relationships between the Public Analysts and the Laboratory of the Government Chemist had been established and persist to this day. The analysts organised themselves as "The Society for Public Analysts" in 1874 (Dyer & Mitchell 1932) to establish and maintain quality standards for food and professional standards for analysis. The modern equivalents are the 'Analytical Division of the Royal Society of Chemistry' and the 'Association of Public Analysts'.

As the grosser forms of adulteration disappeared in large part owing to the work of the Public Analysts public concern diminished. Oddy concludes that the continuance of [food] adulteration long after it was made illegal [by the 1875 Act] was due to the fragmentary nature of local government, the inadequate provisions of the legislation and the money to be made from adulteration. French and Phillips describe the tension between food safety and food authenticity, arguing that after 1919 the newly formed Ministry of Health focused exclusively on the protection of consumers' health believing that the problems of dangerous adulteration of food had been dealt with by earlier legislation. We shall return to this theme in the examination of food authenticity and the 2013 horse meat episode in Walker, Burns and Burns [20].

3.2.2 Modern regulation of food

Modern regulation of food is harmonised globally through the *Codex Alimentarius*⁸ and across major trading blocs such as Europe. As the 20th century ended, a series of food scandals gave rise to renewed public concern; the BSE epidemic, (Phillips, 2000, Vos 2000), *salmonella* in eggs, and a highly significant dioxin contamination in Belgium in 1999, (Covaci, 2008), resulted in intensified efforts to restore confidence in food safety. These included the formation in the UK of the Food Standards Agency⁹ and a sea-change in European food control law. The circumstances leading up to the formation of the Food Standards Agency are well described by MacDonald and Hume, (2000) who also set out the development of food regulation in the UK.

The new European Union, EU, approach from 2000 onward, 'From the Farm to the Fork', is intended to guarantee a high level of safety for foodstuffs and food products marketed within the EU, at all stages of production and distribution, and involves both food products produced within the EU and those imported from non-EU countries (EU, 2013c). Two key pieces of legislation were enacted. Regulation 178/2002 laid down the general principles and requirements of food law, established the European Food Safety Authority (EFSA) and set up new procedures. Output [26] Walker & Wong, 2014 describes in detail the elaboration of European food law.

Article 8 of Regulation 178/2002 deals with the protection of consumers' interests and requires food law to protect the interests of consumers and provide a basis for consumers to make informed choices in relation to the foods they consume. Food law must aim to prevent:

- (a) fraudulent or deceptive practices;
- (b) the adulteration of food; and
- (c) any other practices which may mislead the consumer.

⁸ The *Codex Alimentarius* or "Food Code" was established by FAO and the World Health Organization in 1963 to develop harmonised international food standards, which protect consumer health and promote fair practices in food trade, <http://www.fao.org/fao-who-codexalimentarius/en/> (accessed 27.07.2016)

⁹ Food Standards Agency, FSA, a non-ministerial government department, headed by a Board, Walker was a founder Board member, <http://www.food.gov.uk/>

Article 14 of 178/2002 makes clear the food safety requirements:

- (1) food shall not be placed on the market if it is unsafe; and
- (2) food shall be deemed to be unsafe if it is considered to be either injurious to health or unfit for human consumption.

Regulation 178/2002 was followed and supplemented by Regulation 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. This Regulation is designed to augment existing legislation on official control of food and feed by a harmonized Community approach to the design and implementation of national control systems. Regulation 882/2004 is intended: to prevent or eliminate risks which may arise (either directly or via the environment) for human beings and animals, or reduce these risks to an acceptable level; and to guarantee fair practices as regards trade in food and feed and the protection of consumers' interests, including labelling of food and feed. Regulation (EC) No 882/2004 has been in application since 1 January 2006. At the time of writing Regulation 882/2004 is under review, (Walker & Wong, [25]).

In the UK the Act of 1875 has been regularly updated, the current equivalent measure, the Food Safety Act 1990, provides the enabling powers under which all food regulations, including those on food labelling, are made. Food in the UK is largely criminal law in which the burden of proof on the prosecution is one of beyond reasonable doubt (MacDonald and Hulme; Law Commission 2009; Diamond 1990). This is translated in analytical chemistry in a number of ways - mainly as a 95% confidence interval of the expanded measurement uncertainty below the mean when appraising a result against a maximum limit. The main criminal offences in the Food Safety Act 1990 are rendering food injurious to health (Section 7), selling, to the purchaser's prejudice, food which is not of the nature or substance or quality demanded (Section 14) and falsely or misleadingly describing or presenting food (Section 15). The General Food Regulations 2004 (as amended) provide for the enforcement of certain provisions of Regulation (EC) 178/2002, including imposing penalties, in Great Britain and amend the Food Safety Act 1990 to

bring it in line with Regulation (EC) 178/2002.¹⁰ Similar legislation applies in Northern Ireland. UK food law and its development are the subject of standard texts such as that of Atwood, Thompson and Willett (2009).

UK arrangements for the official food control system¹¹, i.e. regulation and enforcement of food law, in the UK are complex. Regulation is primarily the activity of the state embodied in the 'central competent authority', the central government department, and encompasses advising Ministers on policy and drafting legislation for approval by Parliament. Regulatory responsibility in the UK for food is currently shared between the Food Standards Agency¹², Defra¹³ and the Department of Health¹⁴, with approximately parallel arrangements in the devolved countries. Enforcement is an activity carried out to ensure compliance with legislation relating to food in each competent authority's area. In the UK enforcement is mainly a function of local government. An appreciation of the full complexity of the official food (and feed) control system can be gained by scrutiny of successively updated United Kingdom Multi-Annual National Control Plans¹⁵ (UK MANCP 2015) that cover the official control systems in respect of 'feed and food law' as defined by Regulation (EC) 882/2004 and animal and plant health and animal welfare.

Public Analysts are appointed to serve local government in a scientific enforcement capacity subject to the requirements of the Food Safety (Sampling and Qualifications) (...¹⁶) Regulations 2013 which are the

¹⁰ The implementation of EU law in UK legislation is described in a series of reviews compiled by Walker and published on the Government Chemist website: Collection Food and Feed Law: legislation review <https://www.gov.uk/government/collections/food-and-feed-law-legislation-review> (accessed 31.07.2016)

¹¹ Usually referred to as the official food and feed control system reflecting a 'farm to fork' approach and that contamination in animal feed can cause problems in the human food supply chain, e.g. BSE and dioxins.

¹² Food Standards Agency, FSA, a non-ministerial government department, <http://www.food.gov.uk/>

¹³ Defra, Department for Environment Food & Rural Affairs, <https://www.gov.uk/government/organisations/department-for-environment-food-rural-affairs>

¹⁴ Department of Health, DH, <https://www.gov.uk/government/organisations/department-of-health>

¹⁵ The current version covers April 2013 to March 2016, https://www.food.gov.uk/sites/default/files/mancp-uk_0.pdf (accessed 07.03.2016).

¹⁶ Made separately in each of the home countries of the UK, for example the Food Safety (Sampling and Qualifications) (England) Regulations 2013

successor measures to their previous equivalents. The regulations specify the qualifications necessary to be a Public Analyst, or Food Examiner and give special meaning to the official term 'Food Analyst' (e.g. Referee Analyst) pursuant to the Food Safety Act 1990. A person is qualified to be a food analyst or a Public Analyst if that person possesses a Mastership in Chemical Analysis awarded by the Royal Society of Chemistry, (RSC 2016). The regulations also govern the taking of formal samples and referral to the Government Chemist for analysis in the event of a dispute.

Thus, Public Analysts continue to act as the scientific basis of the UK's enforcement service where chemical analysis is required. Some also carry out microbiological examination. Official laboratories must employ staff possessing qualifications which are defined by Food Safety (Sampling and Qualifications) Regulations. In addition, Public Analysts must be formally appointed by a local authority. The FSA is responsible for designating the majority of official feed and food control laboratories in the UK according to the National Control Plan, as required by Regulation (EC) 882/2004. However, Article 12 of Regulation 882/2004 allows competent authorities only to designate laboratories that operate, are assessed and accredited in accordance with EN ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories'. There are currently 16 Public Analyst laboratories within the UK, serving local authorities. The Association of Public Analysts is strengthened by another 7 laboratories, 3 located in the Republic of Ireland, 1 in the Isle of Man, 2 in the Channel Islands and 1 in Australia. Some laboratories are private practices, whilst others are local government departments; all provide the same essential high quality service to the community. A complete list of UK Public Analysts' laboratories is available from the Association of Public Analysts.¹⁷ The sustainability of the Public Analyst service is, nevertheless, precarious, as will be discussed in the concluding section of this volume.

The Food Standards Agency established, in this writer's view, an unsurpassed model of openness and transparency in food policymaking. On the other hand, Rothstein in a series of papers between 1999 and 2013 has,

¹⁷ APA, Association of Public Analysts, <http://www.publicanalyst.com/> (accessed 27.07.2016)
Page 19 of 162

taking the UK food safety regime and the Food Standards Agency's response to a number of issues, critiqued food regulatory policy. Rothstein broadly concludes that the potential benefits of engaging the public in decision-making with regard to science-based policymaking was mitigated by a number of institutional factors although it may have had some limited value in improving public confidence in the regulatory regime. (Hood *et al.*, 1999, Rothstein, 2004, Rothstein, 2005, Rothstein, 2007, Rothstein, 2013)

3.3 Career Progression

Michael Walker gained a BSc in chemistry at Sussex University in 1975 and returned home to Belfast to take up a post in the Northern Ireland Public Analyst Laboratory, a private practice with its headquarters in Chester. He studied part-time for an MSc in analytical chemistry at Queen's University Belfast, QUB, under Professor Duncan Thorburn Burns. Going on to satisfy the examiners in the Royal Society of Chemistry's Mastership in Chemical Analysis, MChemA, the statutory qualification in food, drugs and water required to practice as a Public Analyst, he was accepted into partnership in the practice and appointed Public Analyst to the then 26 local authorities in Northern Ireland. He was resident Public Analyst for 18 years in Northern Ireland, (1986 – 2004). The period of his training, qualification and early practice was characterised by almost continuous political instability, civil unrest and paramilitary activity that claimed the lives of over 3000 people and resulted in ('collateral') bomb damage to his laboratory on at least six occasions. Throughout, Walker and his staff maintained a continuous professional scientific service underpinning the enforcement of food and feed safety and authenticity, water analysis and a general civil practice. As the 'troubles' wound down towards the end of the 20th century Walker and colleagues obtained United Kingdom Accreditation Service, UKAS, accreditation for some 25 test methods in his laboratory to ISO/IEC 17025. The precarious nature of its situation next to a well-known 'bomb alley' in central Belfast and its status as a branch laboratory inevitably meant more costly instrumentation was reserved in the practice headquarters in Chester. However, Walker maintaining his link with QUB was able to access advanced

techniques in the Chemistry Department. Walker established a customer focused approach to local authority scientific enforcement of food safety and standards and was recognised for this in Turner Review of Public Analyst services in Northern Ireland in 1999 (Turner and Gorsuch, 1999).

Walker also developed skills in strategy and policy, holding posts as a non-executive Director, for example on the Boards of the Consumer Council in Northern Ireland¹⁸ (1999 – 2004), and the Food Safety promotion Board, FSPB, (Safe food)¹⁹ (1999 – 2002). He was a founder Board member of the UK non-Ministerial Government Department, the Food Standards Agency (2000 – 2004). He served as Chief Executive of Forensic Science Northern Ireland 2004 – 06 and formed his own consultancy company in 2006. His clients include the Laboratory of the Government Chemist, LGC, in Teddington through which his association with Kingston University arose.

The work of the Public Analyst and Referee Analyst often occupies the interface between science and the law and drawing on his experience Walker frequently is called on to give advice to Public Analysts, central and local Government and the food industry on matters of food law, analysis and interpretation. Many of the publications collected in this portfolio reflect problems that have arisen as referee cases or required such advice. Further details on the responsibilities of the Referee Analyst appointed by the Government Chemist are to be found in Walker and Gray [23] however Figure 2 illustrates the essential process.

¹⁸ Consumer Council - an independent government funded consumer organisation, in Northern Ireland, <http://www.consumercouncil.org.uk/> (accessed 28.07.2017)

¹⁹ FSPB, Safe food, Food Safety Promotion Board, an all-island implementation body set up under the British-Irish Agreement, 1998 to promote awareness and knowledge of food safety and nutrition issues on the island of Ireland., <http://www.safefood.eu/> accessed 27.07.2016)

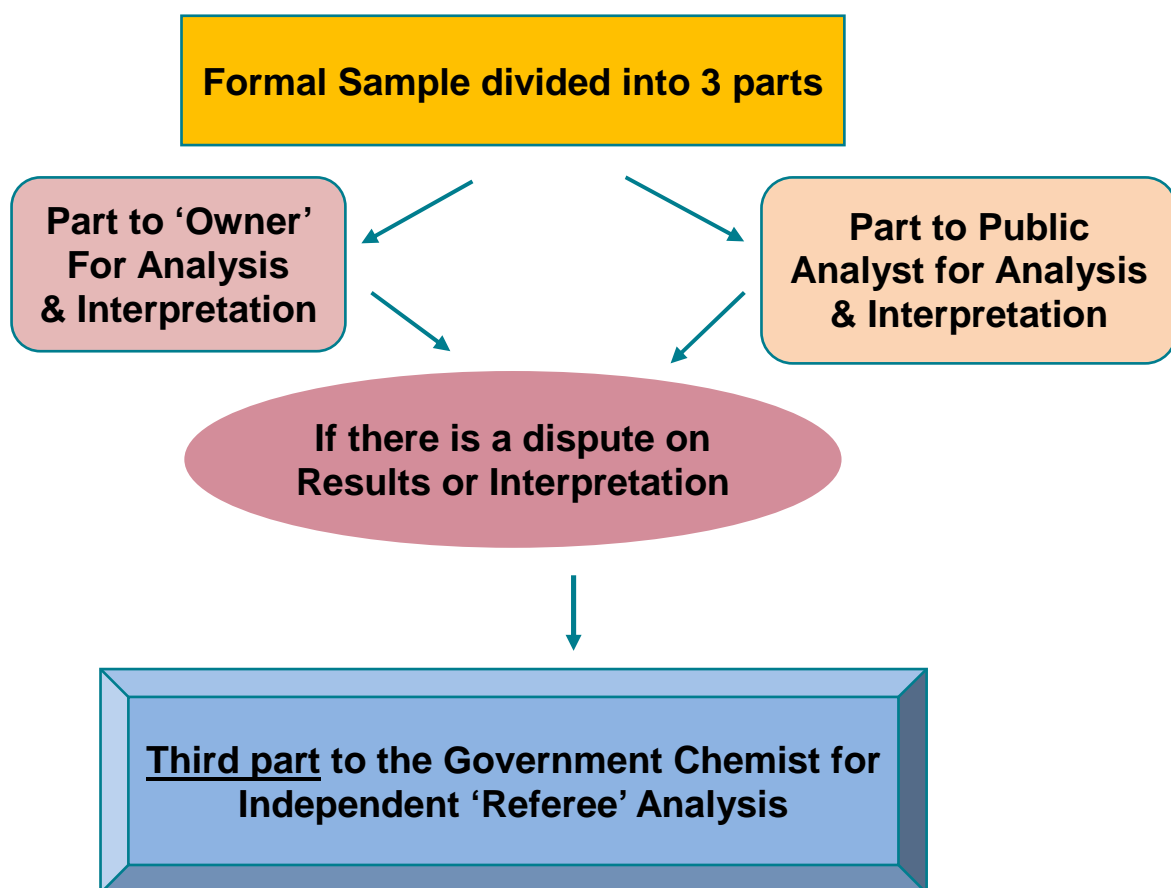


Figure 2: In the UK official food control system only a 'formal' sample can result in legal action if a contravention is uncovered. The formal sample must be split into 3 equal parts and in the event of a dispute there is provision for technical appeal to the Government Chemist.

3.4 Research themes overview

In a busy, often demand-led, career the compilation of this portfolio has given the opportunity to reflect on, articulate and consolidate underlying themes. The subject matter of the work - food additives, food allergens and food fraud (the converse of authenticity) - has been funnelled through the practice of problem solving, learning, sound measurement science, strategic thinking and forensic rigour, Figure 3. The analytical chemistry or molecular biology of additives, allergens and food fraud are described in the context of regulatory protection of consumers and responsible businesses. The *raison d'être* and, it is to be hoped, the outcomes are safer and more honestly labelled food through improvements in analytical science, policy and practice.

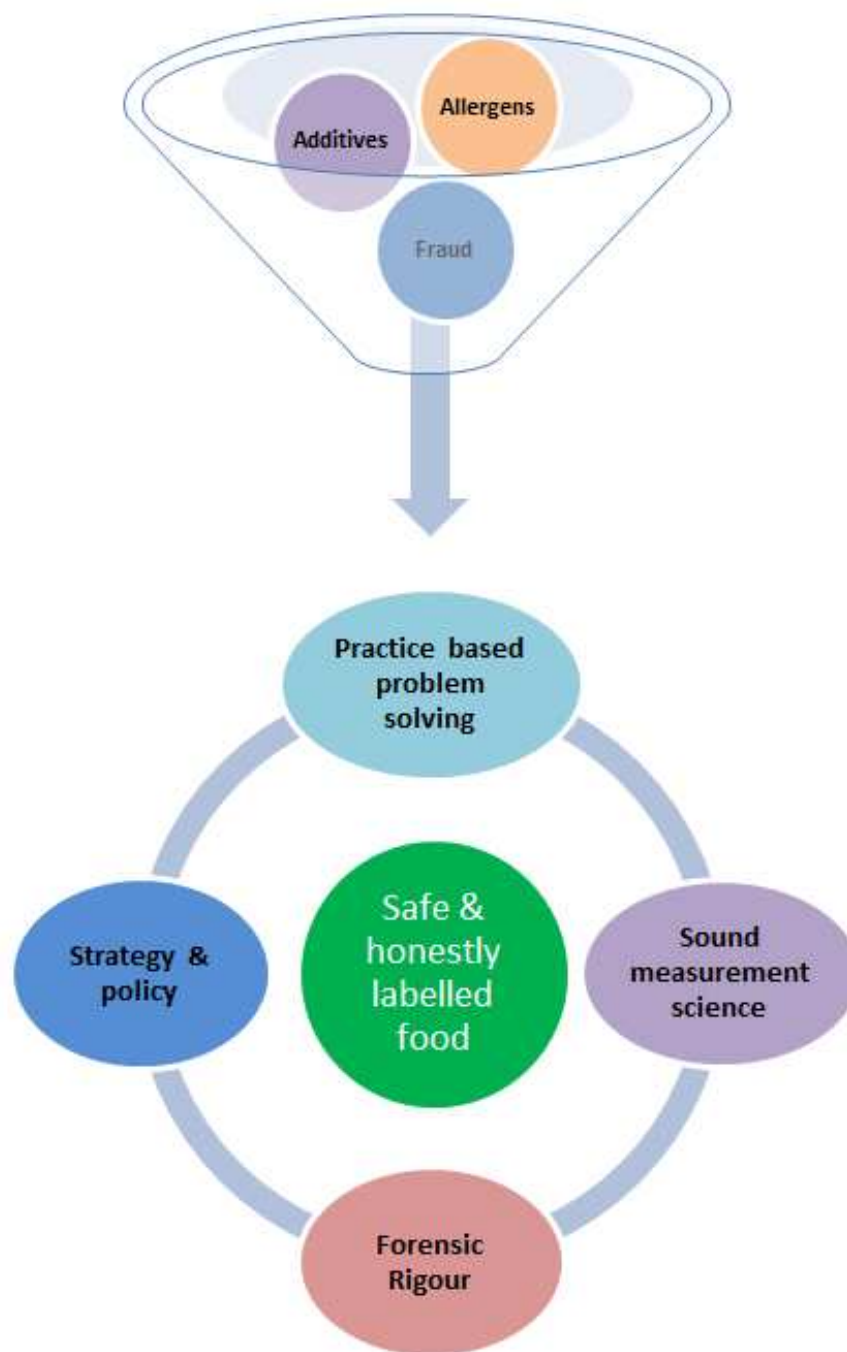


Figure 3: the research themes

The individual sections of Table 1 list the outputs in chronological order. The sections, one with another, possess an underlying coherence, since food additives, food allergens and food authenticity pose differing but very real risks for significant minority population groups, and undermine consumer confidence. Inevitably however the themes exhibit differing chronologies. Indeed papers [1] and [2] from 2009 and 2011 reflect work carried out in

2001 – 2005, collated at that time in unpublished reports. Their publication²⁰ reflected a developing realisation that almost all the valuable data on food generated by Public Analysts was never captured and analysed for trends to establish baselines and inform future work. This notion underpins the work of the Strategic Committee on Food Surveillance in Northern Ireland currently chaired by Walker. The need to protect consumers and responsible businesses including by a sustainable Public Analyst service found expression in Walker's contribution as a Subject Matter Expert to the Elliott Review on the integrity and assurance of food supply networks²¹. Thus the themes mirror significant current health and regulatory issues, reflecting Walker's career development. Figure 4 places the themes and the published work in the context of Walker's career exhibiting, it is suggested, development in depth and breadth of understanding that has led to contributions to the analytical and wider community.

The last part of this introductory section is an overview of the remaining subject matter described more fully below.

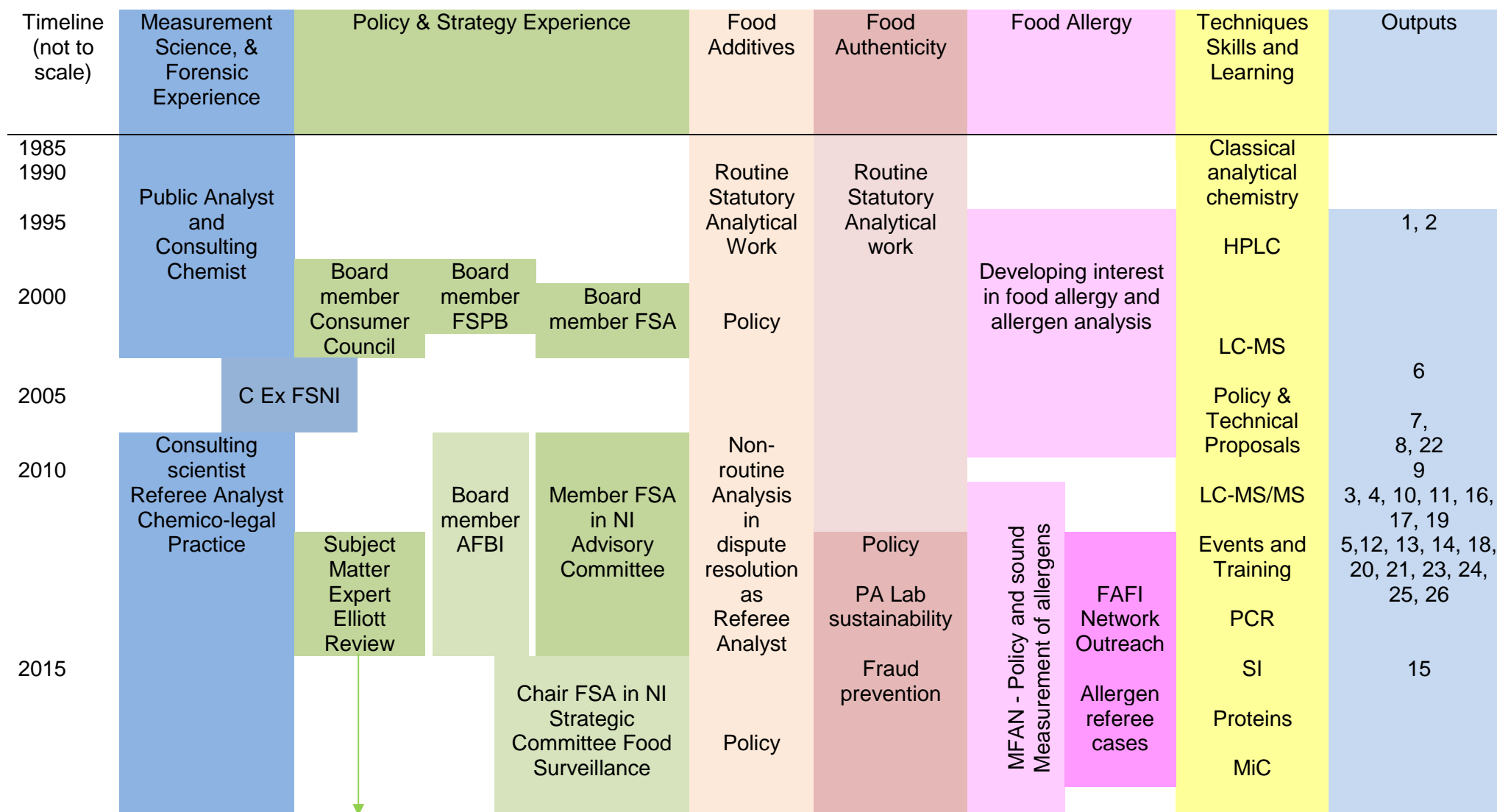
Key to Figure 4:

AFBI	Agrifood Biosciences Institute
FAFI	Food Allergy & Food Intolerance Network, a <i>Safefood</i> funded network run by M Walker 2011 – 15 under contract
FSNI	Forensic Science Northern Ireland
FSPB	Food Safety Promotion Board, <i>Safefood</i>
MFAN	Manchester Food Allergy Network, headed by Professor Clare Mills
MiC	Metrology in Chemistry
PCR	Polymerase Chain Reaction
SI	The International System of Units
Other abbreviations are standard in analytical chemistry	

²⁰ Which owes much to the prompting of Professor Duncan Thorburn Burns

²¹ Elliott Review into the Integrity and Assurance of Food Supply Networks – Final Report, A National Food Crime Prevention Framework, July 2014, published September 2014, available at <https://www.gov.uk/government/publications/elliott-review-into-the-integrity-and-assurance-of-food-supply-networks-final-report> (accessed 25 April 2015)

Figure 4: Walker's Career Progression



3.4.1 Food Additives

The studies on food additives include three key areas:

1. The first publication of previously un-collated additive results, as N.I. Public Analyst, to set a baseline, draw conclusions, inform future regulatory practice and exhort better use of routine analytical results [1, 2]. The work also demonstrated the value of scientific collaboration between both jurisdictions on the island of Ireland which has proved lasting and mutually beneficial. The skills developed included drafting collaborative research proposals, method development and validation in liquid chromatography with diode-array detection, management of potential or perceived conflicts of interest and report writing.
2. Gel forming additives within 'jelly mini-cups' have caused choking fatalities. Chemical analysis of the products in a dispute set out a definitive strategy for their risk assessment and provides the only extant technical guidance on the interpretation of key legislation prohibiting certain additives in jelly mini-cups [3]. The skills developed included physical product testing (size, compressibility, solubility) in relation to human airway obstruction, and integration of the findings with forensic pathology to produce a coherent and workable experimental plan for the assessment of such products. This paper is now widely applied by Public Analysts in their appraisal of jelly mini-cups as choking hazards.
3. Solutions to problems in the analysis of two illegal toxic additives, morpholine and dimethyl yellow, for which forensically robust analytical methods were previously lacking [4, 5]. The skills and learning developed included problem solving in a time constrained scenario and molecular identification by LC-MS to forensic standards (criminal burden of proof). These cases also introduced me to the chemistry of polyoxyethylene ethers²² as it influenced clean-up and chromatography of the target analyte and gel permeation chromatography as a clean-up technique. The limitations included lack of time and funding to carry out full validation studies but the challenge this presented to dissemination of the results for the benefit of the analytical community was overcome.

²² Polysorbate 80 ('Tween 80')

3.4.2 Food Allergens

The work on food allergens has encompassed five interrelated and developing strands of work.

1. The first study to assess in quantitative terms the level of risk to peanut allergic consumers in take-away catering [6] setting the standard for many future such studies and leading to training initiatives [7]. This work published in 2005 was the first research paper I had collaborated on since 1986, introduced me to the problems in allergen analysis and the concept of 'thresholds' – limiting concentrations of food allergens that elicit signs and symptoms of food allergy.
2. The first analysis in Ireland of the awareness of coeliac disease and the gluten status of 'gluten-free' food obtained on request in catering outlets [9].
3. A systematic assessment of allergen analysis [8], a definitive study of the limitations of ELISA in allergen analysis [14] and a programme of improvement and leadership for SI traceable assignment of the concentrations of allergenic proteins in food matrixes, [11, 13].
4. A narrative account of an allergen related food sabotage incident and the subsequent Crown Court case [12].
5. Lastly in this strand are previously uncollated reports of court-sanctions for allergen non-compliances, severe incidents and deaths [10, 15], with policy and practice recommendations to improve the investigation of non-compliant allergen labelling, fraud in the supply chain and food allergen fatalities.

The skills and learning developed included food allergen analysis, metrology in chemistry, basic immunology, protein structures and chemistry, an introduction to exact matching isotope dilution mass spectrometry and reference material production. Recognition as a researcher in the above fields assisted in winning a contract to manage the Food Allergy and Food Intolerance Network for 5 years for *Safefood* – this brought social media and broadcasting skills.

3.4.3 Food Authenticity

Securing food authenticity and preventing its corollary, food fraud, are the *raison d'être* of Public Analysts hence this area of work has been a constant feature of my career. This theme is made up of four strands of work.

1. Collation and critical review of all the then extant data for the species nitrogen content of major commonly consumed animals, fish and shellfish. The species nitrogen concentration ('nitrogen factor') is used as a proxy in the quantitative estimation of high value flesh foods in compound products, a key commercial and consumer attribute and one which is required by law to be disclosed on the label (QUID) [16]. An exemplar study followed determining previously unavailable nitrogen factor data for farmed salmon and salmon frame mince [17]. The study concluded with recommended actions for the more efficient and cost effective gathering of such data in the future with the help of industry.
2. A critical survey of the up skilling of the UK Official Food Control System in DNA food authenticity techniques. This upskilling paved the way for official control work in the horse meat episode [18, 19].
3. A major review of the 2013 horse meat episode in the context of previous such scandals, the law, methods of analysis and their limitations including molecular biology, and UK government food authenticity policy, research and knowledge transfer. The review made policy and analytical recommendations [20].
4. The history of fraud in butter sales critically evaluating traditional and modern analytical authentication techniques; the study reviewed the adulteration of food, to learn lessons from the past, with reference to butter, margarine and fraud. [21].

The skills and learning developed in this research theme included the modern farmed fish and by-product industry, molecular biology and the applications and limitations of nuclear and mitochondrial DNA amplification for quantification of identified species and working remotely with international co-authors.

3.4.4 Chemico-legal practice

This theme covers four strands.

1. Case studies and lessons learned from 'referee analyses' derived from my role as Referee Analyst in the Government Chemist programme [22, 23].
2. Much of my chemico-legal output in the civil and criminal courts, although mainly routine in nature, must remain confidential however one facet has

been published, the estimation, ('back calculation') of blood and breath alcohol concentrations [24].

3. The objectives and basis of European food and feed law, it's development and outworking in the United Kingdom are presented in a book chapter, [25].

4. Metrology in chemistry, a key underpinning discipline safeguarding accurate and precise measurement, is described in a book chapter that also discusses ISO/IEC 17025 the internationally accepted milestone in laboratory accreditation, [26].

These papers encompass mature reflection on my chemico-legal practice.

3.5 Summary

Many threats to our food supply can be assessed and managed only with the assistance of measurement science. This portfolio of work describes novel baseline studies, methods of analysis and means of securing forensically robust findings. Moreover, it includes recommendations for public policy in science and regulation. A final chapter in Volume 1 will be a synthesis of the strands of work on food safety, concentrating on food allergen management, and authenticity with key interconnected learning from each for the benefit of the analytical and regulatory communities and society at large. The mentoring and experience gained in pursuing the studies described in this portfolio, have, I would submit, moulded the author into a well-rounded published scientist, expert witness and public, including media, speaker also comfortable in drafting strategy and policy particularly with regard to food.

The remaining sections of Volume 1 introduce and describe more fully the published work in this portfolio.

4 Food Additives - Discussion

4.1 Permitted Additives

Food additives need little introduction, everyone is familiar with the 'E numbers' in the ingredients lists on food packages. But few food ingredients are more misunderstood. Food additives, including sweeteners and preservatives are strictly regulated in European law. Following the adoption of a common authorisation procedure (European Union 2008a) the European Union list of food additives approved for use in foods and their conditions of use is in Annex II to Regulation (EC) No 1333/2008, (European Union 2008b).²³ No compounds are permitted for use in food as additives²⁴ unless they are assessed independently by EFSA as safe, there is a technological reason for their use and their use does not mislead consumers. In many cases maximum permitted concentrations are prescribed in Regulation 1333/2008. Guidance (FSA 2002) has been made available by successive regulatory authorities on the use of additives and it is the responsibility of manufacturers to ensure their use conforms to legal requirements. Further, analysis by Official Food Control (OCL, Public Analyst) laboratories provides reassurance, given an adequate level of sampling, that additives are not used in foods where they are not permitted, legal limits are adhered to and information required for safety or consumer choice is in fact given. Non-compliances are dealt with including by criminal sanctions.

Despite this protection, consumer concern about food additives persists. The Food Standards Agency has carried out annual consumer attitudes surveys since its inception in 2000. In the years 2000 to 2005 prompted questions yielded concerns about additives from 41% - 45% of respondents. This fell only slightly to 38% for 2006 and 2007 (FSA 2009a). Spontaneously expressed concerns about 'additives/preservatives' were recorded from 5% - 10% of respondents in the latter part of this period and were often at or near the top of such unprompted responses. Consumer attitudes had not changed

²³ Prior to 2008 food additives were regulated by the Miscellaneous Food Additives Regulations (Northern Ireland) 1996 as amended (and equivalents in other parts of the UK). These and previous additive legislation stemmed from European Directives dating from the mid-1960s to the mid-1990s.

²⁴ And given a serial or 'E' number

greatly in 2009 (FSA 2009b), with main issues of spontaneous concern for respondents still featuring the use of additives (11%) with 34% of respondents evincing concerns on prompting. FSA redesigned its consumer surveys in 2010 but in the latest published survey which ran from 5th to the 19th of November 2015 concerns about the use of additives (6 % spontaneous and 20 % prompted were second only to food hygiene when eating out (7 % spontaneous and 28 % prompted), (FSA 2016).

4.1.1 Aspartame and PKU

There are some risks for population sub-groups, however. Consumption of the intense sweetener aspartame (Figure 5) in particular has generated anecdotal reports of conditions including headaches and upset stomachs. The FSA, while reaffirming that aspartame can be consumed safely, initiated a new study in 2009 focusing on people who have reported reactions to aspartame (FSA 2009c). Concerns about aspartame continue (NHS Choices, 2016) but remain to be substantiated. Indeed EFSA, 2013, after a major review of aspartame in which it was noted the compound is rapidly and completely hydrolysed in the gastrointestinal tract to phenylalanine, aspartic acid and methanol, reaffirmed that aspartame is not a safety concern other than to PKU patients.

PKU patients are a small group of people within the population at large who suffer from an inherited error of metabolism, characterised by the complete or almost complete absence of the enzyme phenylalanine hydroxylase. Excess phenylalanine, an essential amino acid, is excreted from the body in a reaction sequence for which phenylalanine hydroxylase is necessary. Accumulation of phenylalanine in the blood leads to a variety of neurological symptoms including mental retardation. The disease is known as phenylketonuria (PKU) and is prevented by mandatory blood screening at birth and dietary measures thereafter to limit the intake of phenylalanine (U.S. National Library of Medicine, 2010). Because aspartame is also a source of phenylalanine all products containing aspartame must be clearly labelled with an indication “contains a source of phenylalanine”, (Regulation 1169/2011, Annex III)..

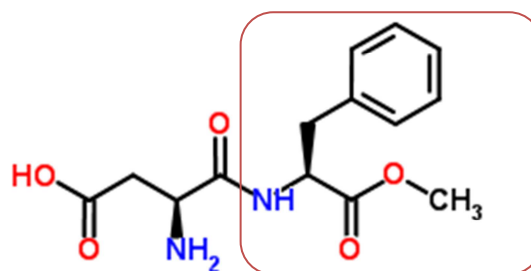


Figure 5: Aspartame, L-Aspartyl-L-phenylalanine methyl ester (Source Chemspider), phenylalanine ester moiety highlighted

4.1.2 Children's behaviour and the 'Southampton' colours

A study commissioned by the FSA (McCann *et al.*, 2007) suggested that a mixture of some food colours and benzoate based preservative could be linked to an adverse effect on the behaviour of hyperactive children. There are now additional labelling requirements for these food colours (FSA 2010, and Annex V Regulation 1333/2008).

4.1.3 Baseline additive data

In view of this continuing interest and in order to place baseline information on the nature and the amounts of intense sweeteners and preservatives in soft drinks on open sale in Northern Ireland in the public domain previously unpublished survey data were collated, Walker *et al.*, 2011 [2].

Although the analysis of a wide range of foods for surveillance and enforcement purposes takes place regularly, the Northern Ireland Public Analysts Laboratory first conducted a survey, with a view to reporting the collated results, into the prevalence of colours and intense sweeteners in various foods (soft drinks, ice cream and takeaway meals) in 1999, (Walker 1999 unpublished). Some soft drinks were found to contain an excessive amount of sweetener and some takeaway meals contained excess colouring matter. It was decided to follow up the soft drinks aspect with a further survey

in 2004 funded by *Safefood*²⁵. The aims were the analysis of up to 150 samples of soft drinks for intense sweeteners (Acesulfame K, aspartame and saccharin) and preservatives (benzoic acid and sorbic acid).

As noted above (§ 3.3) Walker had been a non-executive Director on the Board of the all island Food Safety Promotion Board, FSPB. One of the aims of FSPB is to foster scientific cooperation on the island of Ireland and to that end grants were available to cooperating laboratories from each jurisdiction. A successful grant application was drafted by Walker for funding to develop equivalent methods for intense sweeteners and preservatives in Public Analyst laboratories in both jurisdictions. A post graduate research assistant was then recruited by Walker and directed to carry out the work. The cooperation was secured of the (then) 26 Local Authority Environmental Health Departments in Northern Ireland in conducting a coordinated survey. Operating in a private practice and a former board member of the awarding body it was incumbent on Walker to avoid any perceived conflict of interest and to do so an independent scrutiny committee was established to oversee proper use of the grant funding.

4.1.4 Non-compliances

The published results show the means and ranges of concentrations found were reported for the additives studied. Perhaps unsurprisingly the risk of non-compliance with the maxima permitted in foods was correlated with the mean concentration expressed as a percentage of the maximum permitted concentration. For example for saccharin this was around 75% while for acesulfame K and aspartame the figure was less than 50%. Failure to meet legal requirements was recorded for 14.9 % of samples, and 8.3% exhibited defective or misleading labels. Excess saccharin was found in 2.5%), one sample (0.8%) failed to declare the presence of sucralose, acesulphame K and aspartame, 2.5% had excess benzoic acid and one (0.8%) failed to declare the presence of benzoic acid. All non-compliances were followed up by the Local Authority concerned with the appropriate manufacturer to correct the problems.

²⁵ *Safefood*, the Food Safety Promotion Board, a body responsible for the promotion of food safety in both jurisdictions of the island of Ireland, <http://www.safefood.eu/>
Page 33 of 162

4.1.5 Outcomes – surveillance reports

We suggested that the collection and presentation of data in the manner reported in the paper, now facilitated electronically by the UK Food Surveillance System, UKFSS, (Cree and Reid, 2009) might become a future feature of UKFSS annual reports in Northern Ireland and this is now indeed the case and forms the basis for prioritisation of future work.²⁶

4.1.6 Analysis

The analysis of food additives is well documented (see for example Wood *et al.*, 2004). The additives surveyed in the above work were determined by a procedure obtained in skeleton form from the Public Analyst Laboratory in Galway, Republic of Ireland, in return for mutual assistance with aspects of dioxin analysis. The validation of the method is described in the first paper in this section, Walker *et al.*, [1]. The sweeteners Acesulfame K, aspartame and saccharin were determined in soft drinks and similar beverages, using reverse phase high performance liquid chromatography with a buffered mobile phase and detection by ultra violet absorption detection at 220 nm. This was also a suitable approach for the determination of benzoic and sorbic acids. The method met performance criteria which based on guidance on procedure validation given by the European Medicines Agency. Analytical quality assurance was carried out to the criteria established during the method validation, by 10% replication and participation in appropriate proficiency test rounds. Samples in which excess additives were found were each re-analysed on a separate occasion for confirmation purposes.

4.2 Non-permitted food additives – gels, morpholine & dimethyl yellow

The following three studies were triggered by cases that came to the Government Chemist for resolution.

²⁶ See for example the eighth report on food sampling activity in Northern Ireland in 2014, published by the Northern Ireland Strategic Committee on Food Surveillance chaired by Walker, available at <https://www.food.gov.uk/northern-ireland/news-updates/news/2015/14469/northern-ireland-food-surveillance-sampling-report-published> (accessed 01.08.2016)

4.2.1 Additives as an acute health risk – choking on jelly mini-cups

Jelly cup, or jelly mini-cup, products (Figure 6) first came to prominence in the EU in 2003 with instances worldwide of children and elderly people choking to death on soft slippery dome shaped jellies that were designed to be placed in the mouth in one bite, (Seidel and Gausche-Hill 2002; FSA 2003; Qureshi and Mink 2003; FSA 2004; Anton *et al.*, 2004). The inclusion of certain food additives that foster the production of firm gels represents a hazard if the formed gels become lodged in the airway.



Figure 6: typical jelly mini-cups

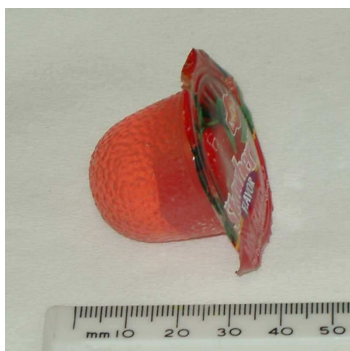


Figure 7: the original konjac-containing jelly mini-cup

The original products (Figure 7) contained 'konjac', E425, a glucomannan food additive that forms a gel that is difficult to dissolve. Konjac was banned in food and manufacturers reformulated jelly mini-cups with other gums with the intention that the sweets could be dissolved in the mouth more easily. However these also give rise to the formation of firm gels that do not solubilise easily. This problem was addressed in the European Union originally by the provisions of Directive 2006/52/EC prohibiting the use of a range of gel-forming additives in jelly mini-cups. The prohibition was carried

over and reinforced in Annex II of Regulation 1333/2008 which also provides the definition of a jelly mini-cup²⁷:

“The substances listed under numbers E 400, E 401, E 402, E 403, E 404, E 406, E 407, 407a, E 410, E 412, E 413, E 414, E 415, E 417, E 418, E 425 and E 440 may not be used in jelly mini-cups, defined, for the purpose of this Regulation, as jelly confectionery of a firm consistence, contained in semi rigid mini-cups or mini-capsules, intended to be ingested in a single bite by exerting pressure on the mini-cups or mini-capsule to project the confectionery into the mouth; E 410, E 412, E 415 E 417 may not be used to produce dehydrated foods intended to rehydrate on ingestion. E425 may not be used in jelly confectionery.”

Unfortunately, the definition given in the Directive of what exactly constitutes a ‘jelly mini-cup’ remains open to interpretation. It is usually straightforward to decide if the product is “contained in semi rigid mini-cups or mini-capsules” but what constitutes “firm consistence”, and whether or not the product is “intended to be ingested in a single bite by exerting pressure on the mini-cups or mini-capsule to project the confectionery into the mouth” remain contentious. In 2008, a dispute arose in the UK between a food importer and a Port Health Authority (PHA) on whether or not a consignment of jelly mini-cups fell within the definition and the matter was referred to the Government Chemist.

The original work (Anton *et al.*, 2004) some of which was carried out within LGC was the starting point for the bench work that decided the issue. Walker planned and, with the assistance of staff in LGC, carried out a series of investigations and had the benefit of discussing the findings with the Northern Ireland State Pathologist, Professor Jack Crane. The outcome was a report that considered each of the defining criteria for a ‘jelly mini-cup’ and upheld the Public Analyst’s findings that the consignment in question did fall within the legal definition and was thus prohibited from entering the food chain. The analytical strategy, the determination of characteristics such as size, weight, compressibility and solubility (or insolubility) in artificial saliva under defined conditions, was subsequently published, Walker *et al.*, 2012 [3]. The paper contains the details needed by any laboratory to apply the various aspects of

²⁷ The definitions in Directive 2006/52/EC and in Annex II of Regulation 1333/2008 are almost identical.

the analytical strategy the sequence in which it should be applied and criteria to make a decision to determine if a product falls within the definition and thus represents a potential choking risk. This paper remains (as of 2016) the sole guidance on the interpretation of the regulations and is widely used by Public Analysts in their assessment of such products.

Walker has been consulted informally on a number of occasions by both Public Analysts and the trade on the published analytical strategy and in 2015 was officially requested to adjudicate on a series of different Public Analysts' reports advancing apparently contradictory opinions on different samples of the same jelly product. This exercise confirmed that the literature is sparse on Jelly Mini-Cups. Since Walker *et al.* 2012 there have been only two papers to our knowledge that consider the matter, both from an interpretational and legal point of view, (Kawawa, 2013, and Kim, 2014). No formal sample having been taken there was no retained portion to forward to the Government Chemist and Walker based his findings on the reports of the Public Analysts, concluding that the owing to the chemistry of one of the gels, carrageenan, and other factors the products probably were not manufactured to a consistent quality. Although advancing the view that on balance the product line was likely to fall within the legal definition of a jelly mini-cup, Walker remitted the issue to the Local Authority for formal sampling and analysis by a third Public Analyst. This would have allowed the trader to obtain their own analysis and interpretation and if it differed from that of the Public Analyst the retained part of the sample could come to the Government Chemist. In the event the third Public Analyst also concluded that the product fell within the legal definition of a jelly mini-cup, this was accepted and the consignment was rejected.

This latter enquiry also gives us an opportunity to correct a misapprehension in our original paper that there had been no UK fatalities. In fact in 2003 in Bolton an 18 month old boy died with the inquest told by the pathologist that "two teaspoons of the jelly [were found] blocking the baby's throat." Recording a verdict of accidental death, the Bolton coroner said: "He had a chest infection but it is my belief that the largest cause of his death was the sweet. I

hope the family can draw some comfort from the fact that as a result of this loss other children will be protected”,²⁸.

It is intended to publish a further paper on jelly mini-cups to complement the original paper.

4.2.2 Morpholine

Morpholine, (Figure 8) is a cyclic secondary amine ether not permitted as a food additive in the UK or Europe.

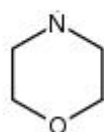


Figure 8: morpholine

In October 2010, the UK Food Standards Agency, FSA, revealed that morpholine had been found on some apples imported from Chile into the UK. Food business operators (FBOs) were advised that apples treated with wax containing morpholine should not enter the UK food supply. An importer sought to challenge the finding by a Public Analyst of morpholine in his consignment of apples by appeal to the Government Chemist. Although the importer subsequently decided to re-export the consignment the Local Authority involved was keen that the Government Chemist should conclude the investigation. Walker and the Public Analyst shared this view as, in discussion, both agreed that with no official method for morpholine in fruit available in the UK the methods used based on derivitisation and liquid chromatography with UV detection risked false positives. Accordingly a literature review and a series of experiments were planned.

Food can be contaminated with morpholine through direct treatment of fruit with waxes containing the compound, through steam treatment during processing and from packaging (IPCS 1995; NIST 2008; NLM 2011;

²⁸ Wigan Today, 22 Sept 2003, <http://www.wigantoday.net/news/local/new-scare-on-sweets-1-205766> and Manchester Evening News 9 August 2004, <http://www.manchestereveningnews.co.uk/news/greater-manchester-news/sweets-ban-after-toddler-dies-1136270> accessed 26/05/2015

Hotchkiss and Vecchio 1983). For example morpholine is listed by the FDA as a component of protective coatings applied to fruits and vegetables as a salt of one or more fatty acids, and as a permitted boiler water additive giving up to 10 mg kg⁻¹ in steam except in steam in contact with milk or milk products (US FDA 2011).

Morpholine is a precursor of carcinogenic nitrosamines (IPCS 1995; NLM 2011; Thorburn Burns and Alliston 1971) and an irritant, for which exposure limits in workplace atmospheres are prescribed by the UK Health and Safety Executive, (HSE 2007). Numerous methods are available to monitor workplace atmospheres and its use as a corrosion inhibitor in water, and in pesticide residues. The more recent methods are based on gas chromatography-mass spectrometry (GC-MS) or liquid chromatography using mass or UV spectrometric detectors, (LC-MS, LC-UV), (Maizels and Budde 2001; Lindahl *et al.*, 2001; Akyüz and Ata 2006; Paik *et al.*, 2006; Fournier *et al.*, 2008; Akyüz 2008). Studies of the occurrence of morpholine in food have been limited but include determination by gas chromatography after extraction and derivatisation with p-toluenesulphonyl chloride (Singer and Lijinsky 1976), with benzenesulfonyl chloride [Hamano *et al.*, 1981; Pfundstein *et al.*, 1991) or with diethylchlorothiophosphate (Kataoka 1995).

Only one previous publication was found for the determination of morpholine in apples, (Sen and Badoo 1980), who used gas chromatography and a thermal energy detector after prior conversion to *N*-nitrosomorpholine (NMOR), giving a detection limit of 0.5 µg kg⁻¹. Following a series of simulation experiments they found that the possibility of the formation of NMOR in the human stomach, after ingestion of such wax-coated apples, is highly unlikely

Walker and his team investigated extraction followed by (a) LC-MS, which proved insufficiently sensitive, (b) LC-MS of dansylated morpholine. The latter yielded good separation and high sensitivity but exhibited mass spectrometric fragment ions predominantly originated from the derivatising group rather than the morpholine moiety. Against the criminal burden of proof and chemical opinion on confirmation preferably with orthogonal selectivity

(Lehotay *et al.*, 2008) Walker did not consider the lack of mass spectrometric confirmation of the chromatographic separation to be sufficiently forensically robust. Hence an amine acetylation (Figure 9) derivatisation method (Bosin and Faull 1988) was proposed from which fragment ions originating from the morpholine group were detected using widely available GC–MS. With full validation, a forensically robust confirmation of the presence of morpholine via its N-acetyl derivative would be possible in support of regulatory analysis, [4].

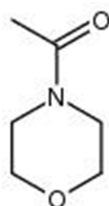


Figure 9: N-acetyl morpholine

4.2.3 Illegal dyes - dimethyl yellow

In 2005 the UK experienced one of its largest food recalls owing to the presence of a Sudan I dye (Figure 10) in chilli powder (FSA 2007). The Sudan Dyes, including dimethyl yellow, have been reviewed (EFSA 2005) and are viewed as genotoxic and/or carcinogenic. Hence they are not permitted to be present in foods at any concentration and are not on the positive list established by Regulation 1333/2008 (see above § 4.1).

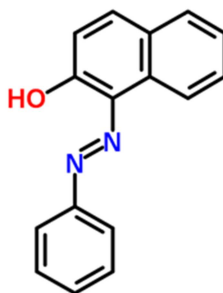


Figure 10: Sudan I, (Source Chemspider)

The presence of illegal dyes such as the Sudan reds in spices is a well-recognised problem, and numerous methods of analysis have been described for their determination (Walker and Stuart 2006, Walker and Stuart 2006a,

Yoshioka and Ichihashi 2008; Feng *et al.*, 2011, and Gray *et al.*, 2016 and references therein).

A problem in analytical methodology for the detection and determination of dimethyl yellow (Figure 11) in turmeric oleoresin with surfactant was recognised following sampling and analysis for official controls in the UK in 2010. Diverse results were reported for 3 sub-samples from a single official bulk sample of a product labelled “Turmeric 15%”. The first laboratory, using LC-UV, found 172 mg kg^{-1} , a result well in excess of an “action limit” for illegal dyes in foods set at $500 \text{ } \mu\text{g kg}^{-1}$ (SCoFCAH 2009,) which if correct would have led to the immediate removal and destruction of the product. However two other laboratories examined the material using LC-MS/MS, with negative results but reported discordant detection limits, namely $2.5 \text{ } \mu\text{g kg}^{-1}$ and $200 \text{ } \mu\text{g kg}^{-1}$. Moreover one of these laboratories reported the need to decontaminate the LC-MS/MS instrument following the analysis, incurring significant down time and the problem was referred to the Government Chemist.

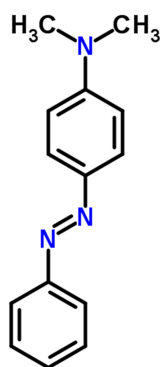


Figure 11: Dimethyl yellow, (*Source Chemspider*)

Walker and his team recognised that the problem lay with the matrix which was identified through trade documents as a turmeric oleoresin. The sample received consisted of an orange-black thick viscous liquid.

Oleoresins are complex extracts of spices. Turmeric oleoresins are produced by the extraction of root turmeric powder by solvents such as acetone, ethanol or dichloromethane (JECFA 1989). The extracts contain natural pigments, curcumins, together with turmeric oil, a complex mixture containing tumerones, sesqui-terpenes, zetenes, phellandrene and cumene (WHO 1999,

Raina *et al.*, 2002). Oleoresins are used in the formulation of oriental sauces, pickles and processed spiced meals and hence likely to penetrate far into the food chain. In the analysis for illegal dyes in an oleoresin the presence of interfering natural pigments has been recognised and dealt with (e.g. by transesterification, Uematsu *et al.*, 2007). But to compound the issue, the product documentation also disclosed the presence of polysorbate 80 (Tween 80), a mixture of polyoxyethylene ethers of mixed partial oleic acid esters of sorbitol anhydrides and related compounds, and an authorised food additive (E 433, polyoxyethylene (20) sorbitan oleate). Significantly, it is a yellow oily liquid. The lipophobic polyoxyethylene ether moiety of its structure (Figure 12) clearly dominates its polarity and solubility characteristics; it is freely soluble in water and polar solvents but much less so in non-polar solvents (JECFA 1973, GSFA 2011). Its presence appeared to promote reverse phase chromatography on silica preventing the isolation of dimethyl yellow by solid (normal) phase extraction, SPE.

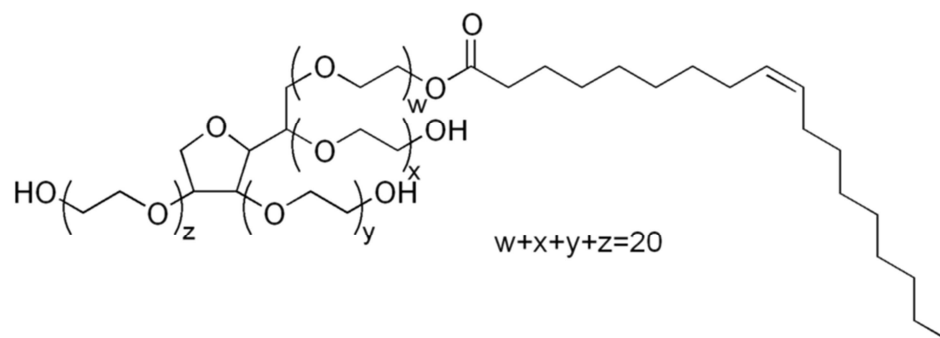


Figure 12: Polysorbate 80, (polyoxyethylene (20) sorbitan oleate)

The problem was solved by the use of gel permeation chromatography, GPC, routinely applied in LGC to remove lipid interferences in pesticides analysis. GPC has been applied to the analysis of illegal dyes (Sun *et al.* 2007, Pardo *et al.* 2009, Oplatowska *et al.* 2011) but not to oleoresin surfactant mixtures. Its use in our hands permitted isolation of a surfactant-free fraction which was amenable to further clean-up by liquid–liquid and SPE prior to LC-MS/MS, which showed it was not forensically credible to report the presence of dimethyl yellow in the referee sample, [5].

5 Food Allergens and Food Allergy - Discussion

5.1 Introduction

Food allergies have resulted in considerable morbidity (Muraro *et al.*, 2014) and reached epidemic proportions in the industrialized world (Prescott and Allen 2011; Sicherer and Sampson, 2014) affecting up to 10% of young children and 2–3% of adults. Anaphylaxis, a rapid onset multi-organ system allergic reaction with release of chemical mediators from mast cells and basophils, can cause fatalities. The risk of such deaths, though comparatively rare, (Umasunthar *et al.*, 2013) contributes to well-documented detriment to the quality of life for allergic consumers and their families, (Avery *et al.*, 2003; King *et al.*, 2009; Venter *et al.*, 2015). There are burdens on health care, (Gibbison 2012) on businesses (food recalls, for example) and regulators (Madsen *et al.*, 2012) and in less developed countries where, owing to poor labelling and awareness, significant challenges may exist. Current reputed cures for food allergies remain experimental and lifelong avoidance of the eliciting food(s) is required. Food intolerance such as coeliac disease also imposes significant burdens [9] and strict food avoidance is usually necessary. A fuller discussion of food allergy is in section 5.3 below.

5.1.1 Gambling your life on a take-away meal

Walker's introduction to the topic of food allergy was in the mid 1990's when, as a Public Analyst, he cooperated with Environmental Health colleagues in producing simple guidance material about nut allergy for food businesses, learning much in the process. In part this activity was prompted by lobbying by the Northern Ireland Anaphylaxis Support Group²⁹. Following this Walker, in cooperation with Dr Ian Leitch, organised a modest 15 sample pilot survey of catering establishments for peanut protein in take-away meals. Leitch had recently completed a PhD on the role of environmental health officers, EHOs, in Northern Ireland in relation to the protection of food allergic consumers. His

²⁹ Northern Ireland Anaphylaxis Support Group ceased to operate sometime after the beginning of the 21st century.

study showed that although EHOs use hazard analysis, HACCP³⁰, as a general method of improving food safety, this approach was not being applied in the control of the allergenic risks. The main reasons were concerns about EHO's lack of knowledge and appropriate training (Leitch *et al.*, 2001). People with food allergy were known to be more at risk when consuming food away from home when the origin and preparation of the food are unknown to its consumer. The pilot survey of peanut protein in take-away meals had three objectives – (a) to determine if take-away curry meals contained peanut protein particularly if requested to be 'peanut-free', (b) to build analytical capability for peanut protein assay by ELISA in the NI Public Analyst laboratory and (c) to stimulate interest in the topic of food allergy to address knowledge gaps identified by Leitch *et al.* This modest initial exercise found peanut protein in 6/15 samples, crucially three of which had been requested to be free of peanut. The method employed was a commercial ELISA³¹ (Besler, M., 2001) and the need to prevent cross contamination at sampling and in the laboratory was acutely appreciated and guarded against.

Success was achieved in all three objectives followed by agreement to fund a larger survey on whether or not it was possible to buy meals suitable for peanut allergy consumers and assesses the training and guidance needs of catering staff and EHOs in Northern Ireland with respect to serious food allergies. The key innovation suggested by Leitch was dual anonymous sampling, initially of a meal likely to contain peanut so as to trigger manipulation of peanut protein in the catering kitchen, and a short time later separate sampling by an EHO from outside the district who asked for a meal for a peanut allergic friend and did not disclose their EHO status until the purchase was made. Information was concurrently gathered about awareness of food allergy in the outlet, how front of house staff reacted to the request and interacted with the customer and the kitchen staff. The EHOs were also surveyed about their knowledge and any guidance or training received on food allergy to gauge the need for future such training. A statistician was

³⁰ HACCP, Hazard Analysis Critical Control Points, (Bauman, 1995), a management system in which food safety is addressed by analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product, originally developed with NASA for meals for manned space flight.

³¹ Tepnel Biosystems Biokits Peanut Protein Test Kit, a non-competitive sandwich enzyme immunoassay, by good fortune one of the more reliable and robust kits then on the market.

consulted on the number of outlets that should be sampled from to represent the known number of take-away outlets in Northern Ireland.

The results were very interesting. All of the initial samples were positive for peanut protein. Of the 62 valid pairs of samples received 13 (21 %) that followed the 'peanut-free' request were positive for peanut protein, 6 (9.7 %) containing > 1000 micrograms of peanut protein. In 7/13 (11.3 %) of sales unfounded reassurance on the safety of the meal for someone with peanut allergy was given by the outlet staff. Most front of house staff did not check the allergen status of the meal with those doing the cooking and most EHOs felt that they needed more training in the subject of food allergen control in commercial food premises. The survey took place in 2002 and was published in 2005 [6]. This paper was the first large-scale baseline study of its kind and influenced a great deal of further similar sampling by EHOs and Trading Standards Officers, and analysis by Public Analysts all over the UK. Walker published advice and guidance on the members section of the Association of Public Analysts, APA, website in 2003. The work stimulated Walker to an appreciation of the difficulties of allergen analysis and the concept of thresholds of elicitation, which in embryonic form were used to interpret the semi-quantitative positive results in the survey. The publication has been cited 26 times by other researchers. Walker's work with the Food Standards Agency 2000 – 2004 assisted in his growing awareness of food allergy, as Board liaison with the Chemical Safety and Toxicology Division, Walker participated in three Food Intolerance Research programme workshops in 2000, 2001 and 2003. These workshops offered opportunities for dissemination and discussion between all the research contractors involved in the FSA research programme on food intolerance.

5.1.2 Food Allergy training

A further positive outcome from the baseline peanut protein survey discussed in § 5.1.1 was a cross border food safety training programme for EHOs funded by *Safefood* and project managed by Dr Ian Leitch. Underpinned by research identifying key areas of need the training marked the beginning of a substantive effort to embed HACCP application to food allergen management

in the catering sector by equipping EHOs, the frontline enforcement professionals, to advise, encourage and enforce in a systematic manner and thus contributing to improvements to the quality of life and safety of food allergic individuals. In 2007 a total of 87 EHOs were trained in 11 workshops held in 7 border counties of both Northern Ireland and the Republic of Ireland. In preparation for the workshops 8 modules of distance learning internet training were offered and the programme concluded with a major training conference bringing together all of the key disciplines involved in food allergen risk management. Walker was not involved in this training programme but won on open tender a short desk based assignment to evaluate the programme development and delivery. The report, [7], was positive, offered recommendations for future work and was well received.

5.1.3 Coeliac disease and 'gluten-free' food in catering

In 2009 Walker was asked by *Safefood* to assist with the evaluation and, if possible, publication of a further baseline survey, this time of the gluten status of 'gluten-free' food obtained on request in catering outlets in Ireland. Walker and Professor Burns were glad to do so on a *pro bono* basis as it afforded an opportunity to learn more about the autoimmune coeliac disease which differs from food allergy (see below). Coeliac disease, CD, is a chronic inflammatory intestinal disease, with debilitating symptoms and potentially serious consequences, induced in genetically susceptible individuals by ingestion of gluten for which the only effective treatment is a lifelong diet that is as free from gluten as possible. Gluten is a generic name for a protein fraction from certain cereal grains containing prolamins (usually estimated as 50% of gluten) and glutenins. Prolamins include the aqueous ethanol soluble proteins gliadins (wheat), secalins (rye), hordeins (barley) and avenins (oats) (Green & Jabri, 2003; Collin *et al.*, 2004; Brottveit & Lundin, 2008; Stazi *et al.*, 2008; McGough, 2009). As with our paper on peanut protein the *Safefood* study provided baseline evidence to inform future interventions of benefit to CD sufferers by examining awareness of CD issues by food servers and preparation staff, assessing their claims to provide gluten-free foods by analysis of a meal concurrently sampled.

Sampling was carried out by EHOs in Northern Ireland and in the Republic of Ireland. Analysis was carried out in the Public Analysts Laboratory in Galway under Dr Andrew Flanagan by 'Mendez cocktail' extraction (Garcia *et al.*, 2005, Lester, 2008; Weber *et al.*, 2009; da Silva Neves *et al.*, 2010) (250 mM 2-mercaptoethanol and 2 M guanidine hydrochloride), followed by R5 monoclonal antibody ELISA (r-biopharm RIDA Screen gliadin kit 96 well sandwich ELISA, LOD gluten 5 mg kg⁻¹, duplicate wells). Positive findings were confirmed by repeat analysis. For food samples containing chocolate, coffee, cocoa or tannin a fish gelatin solution extractant (Skerritt *et al.*, 1991) was used, (4.5% m/V fish gelatin (e.g. Sigma, No. G-7765) and 2% m/V polyvinylpyrrolidone in 60% ethanol). The concentration of gliadin in each sample was calculated in mg kg⁻¹ and the gluten content of each sample was calculated as gliadin x 2. Analytical quality control included in each run the use of reference materials, both negative for gluten and with a known concentration of gluten. Satisfactory results were obtained in blind external proficiency tests of gluten analysis, (Food Analysis Performance Assessment Scheme, FAPAS®).

The findings were in line with our previous work on peanut protein. While the majority of attempts to purchase a 'gluten-free' meal on request in restaurants were successful, some 10 % of all samples contained gluten, 2.7% between 21 and 100 mg kg⁻¹, and 7.7% >100 mg kg⁻¹ and two unsatisfactory samples were purchased from self-styled 'coeliac-friendly' restaurants. The findings were also consistent with those obtained previously by Storsrud *et al.* 2003, Collin *et al.* 2004 and Ge'linas *et al.* 2008. Staff confidence, 'gluten-free' notices, signs and menu choices were no guarantee of risk-free dining for CD sufferers. In our published paper [9] we suggested the need for further training, specifically for chefs and managers. This was taken forward by Safefood in a 'train the trainer' programme with catering colleges on the island of Ireland.

Issues such as the suitability of oats for coeliac patients (intrinsically toxic or contaminated by wheat) continue to deserve investigation. Gluten analysis exhibits difficulties of ELISA kit specificity (Rosell *et al.*, 2014). Moreover the R5 antibody was patented creating difficulties in specifying an undoubtedly

effective tool for gluten analysis in legislation. The current Type I Codex method for gluten analysis is the ELISA R5 Mendez method but analysts should be free to choose the most effective approach. A more recent ELISA making use of the G12 antibody has been independently compared with the official R5 method and the results were found to be comparable, Hochegger *et al.*, 2015.

5.1.4 Food Allergen analysis

When Walker commenced consulting with the Government Chemist Programme in LGC in 2006 one of his first tasks was to draft a proposal, which was accepted, for funding to build capability in the Programme for allergen analysis. The capability building project has been running since 2008, project managed by Walker, and has enabled the Government Chemist Programme to respond appropriately to several high profile referee cases on allergens. It has generated >11 outputs, papers [8], [10], [11], [12], [13], [14], and [15] in this portfolio, an additional three DNA based publications,^{32, 33, 34}, a critical review of allergen analysis by Walker and colleagues³⁵ and a series of papers on the cumin/paprika/mahaleb issue, one of which has recently been published³⁶. Four further papers are in draft.

Walker's allergen work for the Government Chemist continued with a critical review of the literature (2004 to 2007) on allergen analysis [8]. This review confirmed that ELISA and DNA techniques dominate laboratory testing for allergens. DNA based methods have been criticised because they do not

³² Valdivia, H and Burns, M., 2010, Sample Preparation and DNA Extraction for the Detection of Allergenic Nut Materials, *Journal of the Association of Public Analysts (Online)*, 38, pp.1 - 12

³³ Burns, M., 2013, Identifying allergenic nut species using the QIAxcel® system, application note
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/320120/Identifying_allergenic_nut_species_using_the_QIAxcel_system.pdf (accessed 02.08.2016)

³⁴ Busby, E. and Burns, M., 2014. A Simple DNA-Based Screening Approach for the Detection of Crop Species in Processed Food Materials. *Journal of the Association of Public Analysts (Online)*, 42, pp.035-060.

³⁵ Walker, M.J., Burns, D.T., Elliott, C.T., Gowland, M.H. and Mills, E.C., 2016. Is food allergen analysis flawed? Health and supply chain risks and a proposed framework to address urgent analytical needs. *Analyst*, 141(1), pp.24-35

³⁶ Burns, M., Walker, M., Wilkes, T., Hall, L., Gray, K. and Nixon, G., 2016. Development of a Real-Time PCR Approach for the Specific Detection of *Prunus mahaleb*. *Food and Nutrition Sciences*, 7(08), p.703.

target allergenic proteins and data handling practices remain to be standardised. Published peer reviewed independent validation studies for both techniques were lacking for all but a few allergens, (and this remains the case). The review also unveiled the inklings of what were to be persistent problems with allergen analysis. ELISA kits are available for most but not all major allergens but quantification can be problematic. During 2006, the UK Food Analysis Performance Assessment Scheme, FAPAS® conducted five allergen proficiency tests, PT. The participants for each of these rounds used ELISA kits from several different manufacturers, but results submitted for each round had to be divided into groups by FAPAS depending on the brand of kit used. According to FAPAS “this separation was considered necessary because previous experience in FAPAS allergens tests has shown that results from ELISA kits from different manufacturers are from different populations and hence it is not wise to carry out a single statistical assessment of all of the results”. Figures 13 and 14 (from Owen and Gilbert, 2009) showing the distribution of findings from PT rounds for sesame in cereal and for hazelnut in chocolate further illustrate the wide variation of allergen results typically obtained. The situation is not much better now.

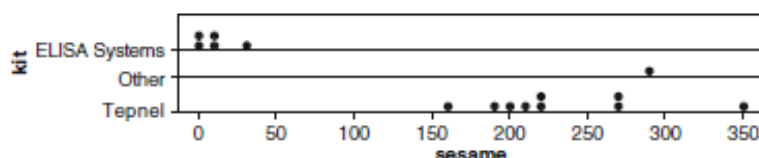


Figure 13: Dotplot showing wide variation of sesame (mg kg^{-1}) from a proficiency test round run by FAPAS in 2006 (Owen and Gilbert, 2009)

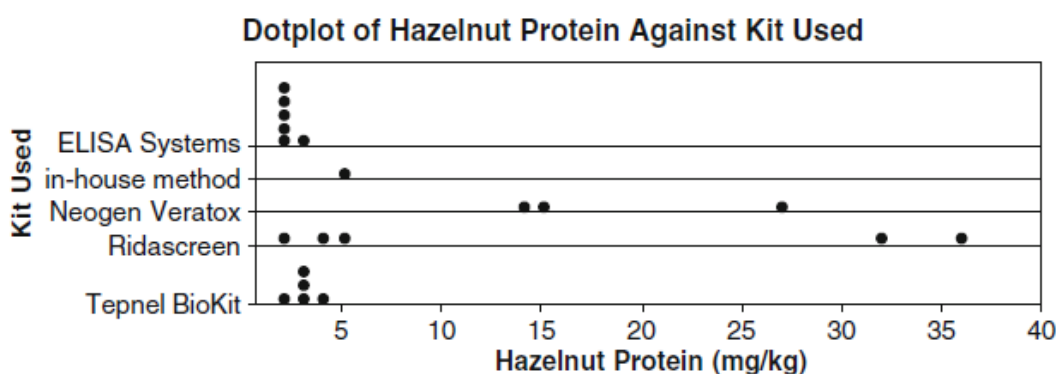


Figure 14: Dotplot showing wide variation of hazelnut as hazelnut protein from a hazelnut proficiency test round run by FAPAS in 2008/09, (Owen and Gilbert, 2009)

The review also elicited areas of good practice, a list of 8 topic areas and common problems that must be addressed when evaluating and validating kits and methods. The review concluded by identifying liquid chromatography coupled with mass spectrometry as a powerful confirmatory technique that with growing databases of allergenic protein amino acid sequences showed promise for allergen identity confirmation. The review thus provided the priorities for the subsequent work in the capability building project.

5.1.5 Analysis – mass spectrometry

Mass spectrometry is a powerful technique for the identification of proteins in complex matrices and it is also considered applicable for quantification of proteins leading to results traceable to the International System of Units (SI) and the production of certified reference materials (CRM). The assignment of the SI traceable concentration of allergenic proteins in food matrices and the production of CRM would be a major advantage in facilitating standardisation of allergen analysis. Mass spectrometry of allergen proteins requires deep insight into protein chemistry and structure. Fortunately Walker found ready and knowledgeable collaborators within LGC and a major output was the work on lysozyme, [11], [13]. To obtain reliable and comparative results by mass spectrometry the bias of methods must be understood. Homologies in protein sequences have to be considered as do post-translational modifications induced by the manufacturing process. Furthermore for a mass spectrometry platform to be used correctly for quantification of allergens leading to results traceable to the SI, appropriate selection of internal standards, their stability and equilibration in the matrix must be evaluated, (Arsene *et al.*, 2008; Quaglia *et al.*, 2008; Pritchard *et al.*, 2009;; Quaglia *et al.*, 2010).

With egg and milk as the most prevalent childhood allergens and thinking that the well characterised (e.g. Fiedler 1998) egg allergen protein lysozyme in wine would be a suitable model work was carried out on a feasibility study spiking wine with $\sim 1 \text{ mg kg}^{-1}$ lysozyme followed by quantification by proteolytic digestion and exact-matching isotope dilution mass spectrometry, IDMS, (Figure 15).

A concentration of lysozyme in wine of $0.95 \pm 0.03 \text{ mg kg}^{-1}$ was calculated based on the concentrations of two signature peptides, confirming that this type of analysis is viable at allergenically meaningful concentrations. The challenges associated with this promising method were explored; these included peptide stability, chemical modification, enzymatic digestion, and sample clean-up. The method was thought suitable for the production of allergen in food certified reference materials, which together with the achieved understanding of the effects of sample preparation and of the matrix on the final results, was aimed to assist in addressing the bias of the techniques routinely used and improve measurement confidence. Confirmation of the feasibility of MS methods for absolute quantification of an allergenic protein in a food matrix with results traceable to the International System of Units was a step towards meaningful comparison of results for allergen proteins among laboratories. This approach is needed underpin risk assessment and risk management of allergens in the food industry by the use of thresholds or action levels if adopted *and* regulatory compliance with any adopted thresholds (see below § 5.3.13, 5.3.14 and 8.4). The proof of concept was achieved and the intention was to spike the quantified lysozyme solution into solid matrices as a prototype reference material, however it was subsequently discovered that poor stability of the quantified lysozyme solution as prepared in glass vials would not allow that aspect to be pursued.

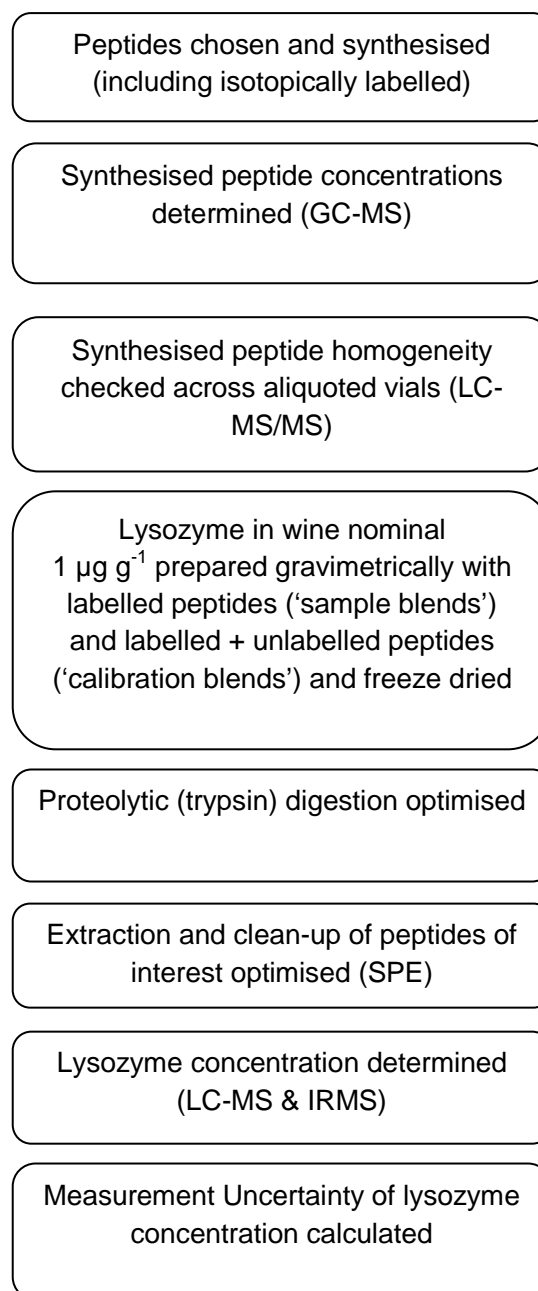


Figure 15: Flow diagram absolute quantification lysozyme in wine

5.1.6 Analysis - ELISA ring trial

The problems of ELISA analysis of food allergens were further highlighted in work spearheaded by Professor Clare Mills, University of Manchester, who founded and runs the Manchester Food Allergy Network, MFAN. This network brings together all the global allergen ELISA kit manufacturers,

retailers, food manufacturers, toxicologists and regulators. Walker attends as an analytical chemist working in the allergens field and has brought in Public Analysts in the UK and the Galway Public Analysts Laboratory, the Republic of Ireland's centre of expertise in allergen and gluten analysis. The aims of MFAN are to improve allergen analysis and explore avenues for better allergen management. MFAN is essentially a facet of Professor Mill's much larger research programmes, iFAAM³⁷ and its predecessor EuroPrevall³⁸.

This work [14] was a ring trial of allergen measurement capabilities by all the available ELISA kits in the hands of laboratories accustomed to using them (or had become so in a pre-ring trial). The test material was based on the dessert matrix (cold swelling starch and other ingredients) previously in EuroPrevall to blind dosage forms used for diagnosis of food allergies, (Cochrane *et al.* 2012). The dessert matrix was incurred with pasteurised egg white or skimmed milk powder at 3, 6, 15 and 30 mg allergen protein per kg of dessert matrix and circulated for allergen analysis in a multi-laboratory trial. Analysis was performed by immunoassay using five kits each for egg and milk (based on casein) and six 'other' milk kits (five based on β -lactoglobulin and one total milk). All kits detected allergen protein at the 3 mg kg⁻¹ level. Based on ISO criteria only one egg kit accurately determined egg protein at 3 mg kg⁻¹ ($p = 0.62$) and one milk (casein) kit accurately determined milk at 6 ($p = 0.54$) and 15 mg kg⁻¹ ($p = 0.83$), against the target value. The "other" milk kits performed least well of all the kits assessed, giving the least precise analyses. At the suggestion of Walker it was also concluded that the incurred dessert material had the characteristics required for a quality control material for allergen analysis.

Recognising that a well characterised quality control material, ideally a certified reference material, has been called for by many to improve

³⁷ Integrated approaches to food allergen and allergy management (iFAAM) aims to: Develop evidence-based approaches and tools for management of allergens in food; Integrate knowledge derived from their application into food allergy management plans and dietary advice; Develop strategies to reduce the burden of food allergies in Europe. <http://research.bmh.manchester.ac.uk/iFAAM> (accessed 03.08.2016)

³⁸ The main objective of EuroPrevall was to "examine the complex interactions between food intake and metabolism, immune system, genetic background and socioeconomic factors to identify key risk factors and develop common European databases". European Commission, http://cordis.europa.eu/result/rcn/51771_en.html (accessed 03.08.2016)

consistency in analytical results for allergens Holcombe and Walker went on to commercialise the study matrix hence maintaining a relationship with the concentrations that affect those with food allergy. They addressed the practical difficulties with production, including ensuring sufficient homogeneity and long term stability. The prototype quality control set, (a blank material and a QC material with peanut protein added at 10 mg kg⁻¹) was prepared and is now available to the wider analytical community as evidence of progress in addressing the lack of allergen related incurred reference materials to improve global allergen protein measurement,^{39, 40, 41}.

5.2 Forensic implications: Food sabotage – a Crown Court and other cases

5.2.1 Food Sabotage

In 2008 Walker was asked to supervise the concluding stages of a laboratory investigation of a food sabotage incident and attend court. The food factory manufactured nut-free ready meals for several of the retail multiples. The incident began when peanuts were found scattered in multiple locations in the production area. To guard against the possibility that peanuts had been included in the nut-free meals and dispatched it was decided by factory management to inform the Food Standards Agency, the company's customers, and institute a product recall. It was evident that this was deliberate sabotage and a possible suspect was a fitter who had been asked to remove some inappropriate material from his workshop wall. His locker was searched. His overalls and factory casual clothing were seized and searched. No peanuts were found in the pockets and the clothing was locked in the Production Manager's office along with the peanuts that had been found in the food handling area. It was now obvious that this was not a simple disciplinary matter and the management decided to call in the police who arrived, took statements and bagged up the evidence. The fitter was arrested

³⁹ **Michael Walker**, Gill Holcombe , Deborah House , Joanna Topping , Clare Mills, 2015, A peanut quality control material to improve allergen analysis – How difficult can it be?, *Clinical and Translational Allergy* 2015, 5(Suppl 3):P116. A fuller paper is in preparation.

⁴⁰ See also poster at http://www.lgcstandards.com/medias/sys_master/root/hf8/h80/8796219277342.pdf (accessed 17.08.2016)

⁴¹ LGC Standards, LGCQC101-KT - Peanut allergen in chocolate dessert positive & negative control <http://www.lgcstandards.com/GB/en/Peanut-allergen-in-chocolate-dessert-positive-negative-control/p/LGCQC101-KT> (accessed 17.08.2016)

and the exhibits of his clothing and the seized peanuts were sent for forensic examination to LGC the forensic supplier to the police force dealing with the inquiry. It was hoped that DNA would be recovered from the peanuts pinpointing who had handled them. However despite extensive swabbing and PRC no recoverable DNA was found and this avenue of investigation proved fruitless. LGC Forensics asked their food science colleagues to look for traces of peanut protein in the pockets of the overalls and clothing belonging to the defendant. The analysis and evidence recovery were undertaken in a dedicated restricted access 'special projects' containment suite, adopting anti-cross-contamination measures as advised by Walker.

The exhibits consisted of 5 sets of overalls, one allegedly from the defendant and 4 from other workers at the plant. Swabs (e.g. Figure 16) from the garments allegedly associated with the defendant returned positive results for peanut protein. In all 24 swabs were taken from the defendant's garments and 21 were positive for peanut protein by ELISA. The overalls belonging to four other workers were swabbed 25 times all with negative results. The defendant was charged with possessing materials for contaminating goods with intent to cause public alarm, injury and economic loss and with threats to kill. The Crown Court jury trial lasted three weeks in early 2009. These forensic results formed a major part of the trial and were given in evidence by Walker assisted by two colleagues.

Part way through the trial, the defence requested experiments to assess the possibility of contact transfer of traces of peanut protein after handling peanuts with subsequent handling of garments and the effects of hand washing. The defence hypothesised that anyone coming into contact with peanuts and then subsequently handling the exhibits could transfer traces of peanut protein onto the exhibit, thus contaminating them.

Instructed by the Crown, Walker set up experiments to see if handling a peanut transferred sufficient peanut protein to the fingers for it to be picked up from fabric. The results and outcome of the tests indicated that even after brief contact with a peanut, peanut protein was readily transferred to clothing and easily detected by analysis even after 10 successive finger/fabric contacts. It was also found that only rigorous hand washing stopped the

transfer. The defence argued that because it was the same management team who found the peanuts and who seized the clothing, it was reasonable to suggest that the management team had, in searching the pockets of the clothing, had themselves contaminated the clothing with peanut protein. After over eight hours of deliberations, the jury indicated they could not reach a verdict on which all of them were agreed and were discharged by the Judge. Consequently the defendant was found not guilty.

The case raised some key points. Food factory management were (and generally remain) unaware of the precautions required in dealing with an allergen sabotage incident. There are well known procedures in crime scene examination that must be followed in order to preserve DNA evidence. Essentially, allergen sabotage evidence must be collected to the same standards. This is not expected to be widely known to police, crime scene investigators or food plant managers. Even minimal handling of peanuts risks widespread subsequent transfer to other surfaces. Hand washing needs to be to a high standard to remove peanut protein deposited on fingers. These points have implications for food manufacturers and possibly for dermal exposure and sensitisation of infants.

Walker wrote up the case as a book chapter [12] for an undergraduate text book. 'Case studies in food safety and authenticity: lessons from real-life situations' published in 2012. The given format of each chapter was interesting, including standardised headings:

Introduction to the case

How did it all begin? What were the challenges involved? (i.e. a brief outline of difficulties that were faced by the experts involved).

Significance of the case

What is the significance of the pathogen, chemical or agent that caused the case?

Regulatory aspects *Did the case have regulatory aspects or implications and if so, which were the laws involved?*

Economic and market aspects *What were the economic aspects of the case? (Export/import statistics, cost-benefit analysis, disease burden,*

*etc.?).*What were the industrial and market aspects of the case? What impact did it have on consumer perception?

The case history continues

What happened and how the issue evolved over time.

What troubleshooting approaches and laboratory methods were used to resolve the problem?

Resolution and outcomes

What were the outcomes of the case? Do any questions remain to be answered?

Commentary

Your own personal reflections. Does the case highlight any research requirements?

Critical questions for the readers

These heading made drafting the chapter enjoyable and are to be commended.



Figure 16: Swab locations inside the pockets of the defendant's overalls

5.2.2 Food allergy cases in the criminal and civil courts of the UK

One of Walker's long term collaborators is Hazel Gowland who has a severe allergy to nuts and peanuts and has worked at national level with the Anaphylaxis Campaign since its earliest days in 1994. Gowland supports and advises those at risk from severe food allergies, both through personal experience and professional expertise. Her work also involves food suppliers as well as families, schools, food enforcement officers, local and national government, doctors, specialist nurses and dieticians. Gowland has developed accessible e-learning resources and allergy training courses for food handlers in the workplace. She investigates deaths and 'near misses' from food allergy provides expert evidence and undertakes scientific, clinical and consumer research into why and how allergen avoidance may fail and how those at risk may be protected.⁴²

Working in a strand of the Government Chemist capability building programme better to understand the forensic context of food allergy Walker and Gowland collated a series of eight food allergy cases in the UK courts involving fatalities, personal injury or criminal non-compliance with food law. The information gathered was from mainly 'grey' literature sources. This work [10], [15] was the first time such material had been gathered together and analysed and the work has been well received by scientists and the legal profession. The authors suggested there should be central collation of such cases, and a step in this direction has been taken with the announcement in November 2015 by the Food Standards Agency of an FSA and local authorities' database of successful food standards, food hygiene and food safety related prosecutions in England, Wales and Northern Ireland.⁴³

The potentially severe consequences for people with food allergy of contraventions of labelling law have led to enforcement action up to criminal prosecution for what might otherwise be regarded as 'trivial' non-compliance.

⁴² Hazel Gowland, Allergy Action, <http://allergyaction.org/hazel-gowland/> (accessed 03.08.2016)

⁴³ FSA, 2015, announcement <http://www.food.gov.uk/news-updates/news/2015/14644/food-standards-agency-publishes-food-law-prosecutions-database> (accessed 03.08.2016)

Table 2 shows the cases examined. The study concluded there is a spectrum of options to protect food allergic consumers, short of litigation and prosecution by enforcement authorities, including self-policing by the industry and allergen alerts. However the courts remain as a potential backstop and consideration of these cases suggests the following conclusions that would enhance forensic effectiveness for vulnerable consumers.

- Calls for a culture of zero tolerance for food fraud are appropriate (a reference to the Elliott Review).
- Enforcement of food labelling and traceability law can reduce the risk to vulnerable consumers since documented traceability of ingredients can lower the risk of including undesired allergen ingredients.
- Enforcement authorities should accelerate their escalation of action against poor labelling and misleading food description when they pose an allergen risk.
- Thorough investigation of food allergy deaths particularly in the catering / non prepacked sector is required where there seems growing evidence that fraud has infiltrated.
 - a. Such investigations require a tenacious and skilled approach with more widespread awareness that analysis of food for allergens is possible and the need for early realisation for example that samples of the food and/or stomach contents from a post mortem examination will be required to be retained and analysed with due regard for the forensic chain of custody of evidence.
 - b. The supply chain of the meal ingredients must also be rigorously followed up to find out where any adulteration or contamination with the fatal allergen occurred.
 - c. Investigation of the incident should have regard to what advice had previously been offered to the food business by enforcement officers.
 - d. A charge of corporate manslaughter in the case of an allergen related fatality may be available under the Corporate Manslaughter and Corporate Homicide Act 2007.

- Salience of the risks of all food allergens should be maintained by patient support groups, regulators and enforcers (given the influence such salience had on the Appeal Court's views in one of the cases, *Bhamra*).
- Food businesses must guard against short cuts or gaps in allergen management; there are many readily available sources of advice, training and guidance and an up-to-date awareness of these is required by staff at all levels.
- New training and guidance on guarding against unwitting or fraudulent substitution in the supply chain, needs to be developed.
- Skills gaps that have emerged in properly prosecuting fraud cases should be addressed.
- A case in which a baby died in a nursery, the Baby Egan case, catalysed improvements for children with allergies in that whole sector and the deficiencies identified by the prosecution provides a template for best practice for child care nurseries and inspection of same.
- The impact of legislation and a careful consideration of case law appear to place responsibility onto food businesses even for the currently problematic area of allergen cross contamination / cross contact.

As prefigured in our paper, in May 2016 Mohammed Zaman, a restaurant owner, was convicted of gross negligence manslaughter and sentenced to six years in gaol after a jury at Teesside Crown Court was told he swapped almond powder in recipes for cheaper groundnut mix, containing peanuts, despite warnings. This had led to the death of Paul Wilson, 38, a customer with a peanut allergy who was meticulous about his condition and asked for no nuts when staff at the restaurant took his order in 2014. Wilson was found slumped in the toilet at his home having died from a severe anaphylactic reaction.

Table 2 Cases Summary (output [15])

Case	Detail	Law	Outcomes
Case 1 Fatality Inquest, Health and Safety prosecution child care 2002	<i>Milton Keynes Council -v- Jigsaw Day Nurseries Ltd, unreported</i> Thomas Egan 5 months died from anaphylaxis to cow's milk in a day nursery. Parents had told staff not to give him cow's milk. The inquest verdict was 'accidental death contributed to by neglect [by the nursery].' Local EHOs undertook a fatal accident investigation and prosecuted the nursery for a series of management failures.	Health and Safety At Work Act 1974, Section 3 subsection 1	£60,000 fine and £19,000 legal costs, criminal record.
Case 2 Fatality Inquest, Civil Action, Appeal catering 2003	<i>Amjarit Kaur Bhamra (Widow & Administratrix of the Estate of Kuldip Singh Bhamra, Deceased) v Prem Dutt Dubb (T/A Lucky Caterers) (2010) [2010] EWCA Civ 13 CA (Civ Div) (Sir Anthony May [President QB], Wall LJ, Moore-Bick LJ) 20 January 2010</i> Mr Kuldip Bhamra died from egg allergy at a Sikh wedding having consumed a dessert, ras malai. Invitations indicated food would be served under temple rules which exclude egg to which Mr Bhamra had a known food allergy. No food sample was retained for testing. Mr Bhamra's widow succeeded in a civil action against the caterer; an appeal was dismissed.	Tort of negligence, (upheld) Contracts (Rights of Third Parties) Act 1999 (dismissed)	Mr Bhamra's widow was awarded £415,000 plus costs
Case 3 Food Law, prosecution Prepacked food 2009	<i>Telford and Wrekin Trading Standards, unreported</i> Market trader selling imported prepacked chocolates was prosecuted for "placing on the market food that was unsafe namely Milka Frühlingsblumen in that the labelling did not declare the presence of allergenic ingredients namely Almond and Hazelnut in a language easily understood." The ingredients were only labelled in German and French.	Reg 4 (a) General Food Regulations 2004 and Reg (EC) 178/2002 Article 14, (1)	The trader was fined £3500 plus costs, criminal record.
Case 4 Food Law prosecution prepacked food, 2010	<i>Royal Borough of Kensington and Chelsea -v- Whole Foods Market, Fresh and Wild Ltd, unreported</i> A customer who has a severe nut allergy bought a portion of Aubergine Rollatini Spinach checked the label and nuts were not listed in the ingredients or on the label. She bit into it and suffered an allergic reaction and was admitted to hospital for emergency treatment and discharged after 8 hours. Defendants, pleaded guilty at West London magistrates' court on 7 September 2011.	Reg 4 (c) of the General Food Regulations 2004 Regulation (EC) 178/2002, Article 16	Fined £2000, £2,321.03 in costs and a £15 victim surcharge, criminal record

Case 5 Food Law prosecution prepacked food, 2012	<i>Essex County Council -v- Minidelikatesy Kubus Ltd, unreported</i> Food not labelled in English	Reg 4 (a) General Food Regulations 2004 and Reg (EC) 178/2002 Article 14, (1)	Company director fined £870, and company fined £2,250 on the basis of the first offence, costs of £1,350 and a victim surcharge of £120, criminal record
Case 6 Food Law prosecution, catering food, analysis, 2009	<i>Derbyshire County Council -v- New China House Takeaway Restaurant, unreported</i> A peanut allergic customer asked a Chinese takeaway for a meal without peanuts, ate the meal and suffered a severe allergic reaction requiring emergency life-saving treatment and stabilisation in hospital. The remaining food was analysed and found to contain peanut protein at a level of 31 mg kg ⁻¹ . The food business operator was prosecuted for supplying unsafe food by failing to declare the presence of peanuts.	Food Safety Act 1990	Fined a total of £2,660, £1,000 costs, £15 victim surcharge and told to give the diner £200 in compensation, criminal record.
Case 7 Food Law prosecution prepacked food analysis 2011	<i>Hull City Council-v- RK Sweets Ltd, unreported</i> Severe allergic reaction to a South Asian confection, marble laddoo said, when asked, not to contain peanuts when sold at an Indian food festival.	Food Safety Act 1990	Company: £5000 fine, £1661.88 costs Director: £2500 fine, criminal record
Case 8 Food Law prosecution Catering analysis 2011	<i>Cumbria County Council –v- Euro Foods, unreported, Appeal Euro Foods Group v Cumbria County Council [2013] EWHC 2659 (Admin)</i> Euro Foods Ltd had supplied groundnuts (peanuts) instead of almond powder as requested, to another wholesaler who had in turn supplied take-away outlets which themselves had no knowledge that the product they were introducing into their meals was not almond powder. Eurofoods initially found guilty on charges of supplying almond products adulterated with peanuts. It was said that this arose because of the price differential. Euro Foods later successfully appealed the conviction and it appears that the grounds of appeal included the error in law of laying information under <i>both</i> sections 15(2) and 15(3) of the Food Safety Act	Food Safety Act 1990, S15(2),15(3) Magistrates' Courts Rules 1981 Criminal Procedure Rules 2005 onwards	Fined £6,000 with £12,000 costs, quashed on appeal

5.3 Food Allergy – review of current knowledge

5.3.1 What is Food Allergy?

[Much of the text in sections 5.3.1 – 5.3.15 is abridged from Walker's contribution to a book chapter, Food Allergy: Managing Food Allergens, Walker and Gowland, in 'Analysis of Food Toxins and Toxicants' Ed Yiu Chung Wong, Wiley, *in press*]

There is a spectrum of adverse reactions to food (Figure 17). For example if anyone eats food containing a large number of *Salmonella* enterobacteria they will be ill – this is a predictable reaction that holds true for all individuals. Other reactions are not predictable; that is to say, until they happen the person does not know they *will* happen, and not everyone is affected. When those reactions happen almost every time the person eats that food the reactions are 'reproducible'. Reproducible adverse reactions to certain foods are termed 'food hypersensitivity'. A formal definition of food hypersensitivity is: 'objectively reproducible symptoms or signs initiated by a defined stimulus at a dose tolerated by 'normal' subjects' (Johansson *et al.* 2001). Food hypersensitivity can take many forms (see below). The term 'allergy' was introduced in 1906 by Clemens von Pirquet (1874–1929) (Igea, 2013) and 'food allergy' is a hypersensitivity to food protein(s) mediated by the immune system. The human immune system is essential to our survival, producing antibodies that recognise, bind to and aid in the destruction of harmful antigens such as parasites, bacteria or viruses. Antibodies are immunoglobulin glycoproteins produced by plasma cells (white blood cells). There are five main isotypes: IgA, IgD, IgE, IgG, and IgM, (Schroeder & Cavacini 2010). Food allergy is mediated by immunoglobulin E, IgE, discovered in 1967 and first termed 'IgND' after the initials of the patient from which it was taken (Johansson, 2016).

There are two separate processes in the development of food allergy – (a) sensitisation and (b) elicitation of signs and symptoms. In susceptible individuals, sensitisation occurs when an immunological response develops to specific food proteins. Sensitisation (production of allergen specific IgE, sIgE) is possible without symptoms of an allergic reaction hence both sensitisation and elicitation of an adverse reaction on subsequent exposure define food allergy. Thus, in sensitised individuals ingestion of that food

protein causes IgE cross linking by allergenic epitopes with release of potent inflammatory mediators such as histamine from tissue mast cells and peripheral basophils. A good explanation of this process has been given by De Leon, *et al.*, 2007. IgE mediated food allergy exhibits acute onset, generally within 2 hours of ingestion of the provoking food. Symptoms include mild lip tingling, diarrhoea, vomiting, pruritus (itch), asthma, urticaria (hives, a raised, itchy rash), and angioedema (swelling caused by a build-up of fluid). The most severe allergic reaction is anaphylaxis which can be life threatening (see below).

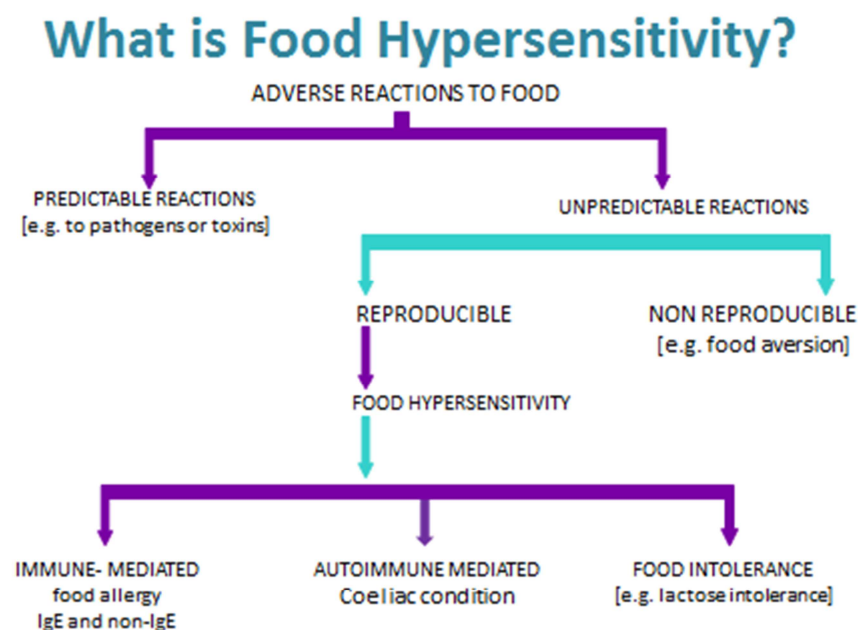


Figure 17: What is food hypersensitivity

Related conditions include oral allergy or pollen-food allergy syndrome, where sensitisation to pollen proteins in the respiratory system results in IgE that binds food proteins in certain fruit and vegetables and intermediate gastrointestinal hypersensitivity.

Mixed IgE and cell mediated mechanisms give rise to chronic conditions such as atopic dermatitis, also known as eczema and, separately, eosinophilic gastroenteropathies, such as eosinophilic esophagitis and eosinophilic gastritis. Non-IgE-mediated gastrointestinal food-induced allergic disorders (non-IgE-GI-FAs) account for an unknown proportion of food hypersensitivity

and include food protein–induced enterocolitis syndrome (FPIES), food protein–induced allergic proctocolitis (FPIAP), and food protein–induced enteropathy (FPE). Non-IgE-GI-FAs have considerable overlap among themselves and with eosinophilic gastroenteropathies. FPIES is probably the most actively studied non-IgE-GI-FA, potentially because of acute and distinct clinical features. FPIAP remains among the common causes of rectal bleeding in infants, while classic infantile FPE is rarely diagnosed. The most prominent clinical features of FPIES are repetitive emesis (vomiting), pallor, and lethargy; chronic FPIES can lead to failure to thrive. FPIAP manifests with bloody stools in well-appearing young breast-fed or formula-fed infants. Features of FPE are nonbloody diarrhea, malabsorption, protein-losing enteropathy, hypoalbuminemia, and failure to thrive. Non-IgE-GI-FAs have a favourable prognosis; the majority resolve by 1 year in patients with FPIAP, 1 to 3 years in patients with FPE, and 1 to 5 years in patients with FPIES, with significant differences regarding specific foods. Much more work remains to be done on these conditions. (Chafed *et al.* 2010; Sicherer and Sampson 2010; Burks *et al.* 2012; Järvinen and Nowak-Węgrzyn, 2013; Caubet *et al.* 2014; Nowak-Węgrzyn, *et al.* 2015). See also Heiner syndrome, milk-induced pulmonary disease in infants (Moissidis *et al.* 2005).

Food hypersensitivity also includes the auto-immune condition coeliac disease (Kennedy and Feighery 2000, McIntosh *et al.*, 2011) or the spectrum of conditions grouped together as ‘food intolerance’. Food intolerance includes pharmacological effects of food components, e.g. vasoactive amines such as histamine, non-coeliac gluten sensitivity, enzyme and transport defects, e.g. lactose intolerance, the potential adverse effects of some food additives e.g. tartrazine, annatto, sulphites, benzoic acid, and short chain fermentable carbohydrates (Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols, FODMAPs) (Lomer, 2015).

However herein we deal mainly with IgE mediated food allergy, an increasing global health problem that can result in considerable morbidity (Prescott and Allen 2011, Nwaru *et al.* 2014; Chan *et al.* 2015a).

5.3.2 Allergen nomenclature

Breiteneder and Chapman recount the inception of systematic allergen nomenclature by Marsh, Løwenstein and Platts-Mills in 1980 (Breiteneder and Chapman, 2014). Originally each allergen name was derived from the first three letters of the genus and the first letter of the species (both in italics) followed by a Roman numeral to indicate the allergen in the chronological order of purification. This was subsequently revised to ordinary typeface and Arabic numerals. Thus as peanut belongs to the genus *Arachis* and the species *Arachis hypogaea* the allergens are Ara h 1, Ara h 2 etc. An allergen nomenclature subcommittee exists under the auspices of the World Health Organization, WHO, and the International Union of Immunological Societies, IUIS, with criteria for including allergens in the systematic nomenclature.⁴⁴

Other common allergens such as cow's milk, from *Bos domesticus*, e.g. Bos d 8 refers to the caseins, and egg, *Gallus domesticus* (chicken) contains Gal d 2, ovalbumin. Allergens may also be categorised according to their protein class, thus Ara h 2 is member of the prolamin protein superfamily. The AllFam database is a resource for classifying allergens into protein families.⁴⁵

5.3.3 Allergy Prevalence

Food allergy may persist from childhood or be a newly acquired adult sensitisation. Some food allergies that start in childhood e.g. to milk, egg, soy, or wheat are often outgrown, whereas allergies to tree nuts or peanut tend to persist. Allergy to fish or crustacean shellfish, which most commonly develops in adulthood, usually persists. Hence the prevalence of food allergy varies, data may be lacking and studies exhibit heterogeneity. The double-blind, placebo-controlled food challenge (DBPCFC), the most reliable indicator of allergy to a food, has proved difficult to apply in many prevalence studies. (Burks *et al.* 2012).

⁴⁴ the website is <http://www.allergen.org/index.php>.

⁴⁵ the website is <http://www.meduniwien.ac.at/allfam/>.

Rona *et al.*, 2007 first identified the main problems in prevalence studies; out of 934 articles identified by these authors from 1990 onwards only 51 were as appropriate for inclusion in their prevalence meta-analysis. Information sources were classified in 5 categories: self-reported symptoms, specific IgE positive, specific skin prick test positive, symptoms combined with sensitization, and food challenge studies. The high prevalence of self-reported food allergy compared with objective measures was also noted.

Nwaru *et al.*, 2013 studied the prevalence and epidemiology of food allergy in 25 countries of Europe in a systematic review of the literature 2000 – 2012. The protocol, search strategy, inclusion and exclusion criteria and key terms were defined. The numbers of new cases of the various IgE-mediated, non-IgE-mediated or combination causes of food allergy that occur during a given period in a defined population were studied as:

- Incidence rate: The number of new cases of food allergy that occur during a given period per unit of person-time;
- Cumulative incidence: The number of new cases of food allergy that occur during a given period per the population at risk;

Prevalence data were collected as:

- Point prevalence: the proportion of the population that has experienced food allergy at a specific time;
- Period prevalence: the proportion of the population that has experienced food allergy during a given period, and
- Lifetime prevalence: the proportion of the population that at some point in their life will have experienced food allergy.

Seventy-five eligible articles (56 primary studies) were included and most of the studies were graded as at moderate risk of bias (Nwaru *et al.* 2014.) There were significant differences between self-reported and other categories. Self-reported pooled lifetime prevalence of food allergy was 17.3% (95% CI: 17.0 – 17.6) accompanied by a self-reported point prevalence of 5.9% (95% CI: 5.7 – 6.1). However the point prevalence of sensitisation to one or more foods also differed with category as shown in Table 3.

Table 3: Point Prevalence to \geq allergen by diagnostic category (from Nwaru *et al.* 2014)

Assessed by	Point Prevalence	95 % Confidence Interval
Specific IgE	10.1 %	9.4–10.8
Skin prick test	2.7 %	2.4–3.0
Food challenge	0.9 %	0.8–1.1

Both self-perception and allergic sensitization (specific IgE) are known to substantially overestimate the actual frequency of food allergy. Overall the data reported by Nwaru *et al.* appear to indicate that food allergy affects some 1 – 2% of adults and some 5 – 6 % infants and children in Europe however more studies are needed. Prevalence was greater in north-western Europe than in southern Europe. While the incidence of FA appeared stable over time, there was some evidence that the prevalence may be increasing.

Prevalence of food allergy to specific foods in Europe was investigated again showing significant heterogeneity in a fewer number of studies, Table 4, (Nwaru, 2014b). Allergy to cow's milk and egg was more common among younger children, while peanut, tree nut, fish, and shellfish were more common among the older ones.

Table 4: Overall pooled estimates for all age groups lifetime prevalence of allergy

Food	Self-reported lifetime prevalence allergy, mean and 95 % Confidence Interval	Lifetime prevalence of food challenge defined allergy, mean and 95 % Confidence Interval
Cow's milk	6.0 % (5.7 – 6.4)	0.6 % (0.5 – 0.8)
Egg	2.5 % (2.3 – 2.7)	0.2 % (0.2 – 0.3)
Wheat	3.6 % (3.0 – 4.2)	0.1 % (0.01 – 0.2)
Soy		0.3 % (0.1 – 0.4)
Peanut	0.4 % (0.3 – 0.6)	0.2 % (0.2 – 0.3)
Treenuts	1.3 % (1.2 – 1.5)	0.5 % (0.08 – 0.8)
Fish	2.2 % (1.8 – 2.5)	0.1 % (0.02 – 0.2)
Shellfish	1.3 % (0.9 – 1.7)	0.1 % (0.06 – 0.3)

Sicherer and Sampson suggested that food allergy in the USA probably affects nearly 5% of adults and 8% of children, with growing evidence of an increase in prevalence (Sicherer and Sampson, 2014).

Mahesh *et al.* reported food allergy sensitisation prevalence (sIgE estimation for 24 common foods) among South Indian adults of 26.5% (Mahesh *et al.*, 2016), but actual food allergy was far less common.

Australia appears to have some of the highest global prevalence of food allergy of up to 10% in young children (Prescott *et al.*, 2013). These authors conducted a global survey in 2012 to collect information from all the national member societies of the World Allergy Organization, and some of their neighbouring countries, (total $n = 89$). More than half of the countries surveyed (52/89) did not have any data on food allergy prevalence. Only 10% (9/89) of countries had accurate food allergy prevalence data, based on oral food challenges, OFC. The remaining countries (23/89) had data largely based on parent-reporting of a food allergy diagnosis or symptoms, recognised to overestimate the prevalence of food allergy.

Food allergy in Asia has been reviewed based on the literature published between 2005 and 2012 (Lee *et al.*, 2013). The overall prevalence of food allergy in Asia was found to be somewhat comparable to that in the West with egg and cow's milk allergy the two most common food allergies among young children and infants. However, by contrast, shellfish allergy rather than peanut allergy is the most prevalent in Asia, in part due to the abundance of seafood in the diet. Lee *et al.* suggest that house dust mite tropomyosin may be a primary sensitizer. Differences also exist within Asia. Wheat allergy, though uncommon in most Asian countries, is the most common cause of anaphylaxis in Japan and Korea, and is increasing in Thailand.

In large and rapidly emerging societies of Asia, such as China there are documented increases in food allergy. The prevalence of oral food challenge (OFC), proven food allergy is around 7% in pre-schoolers, comparable to the reported prevalence in European regions. Comparison of cross-sectional

data collected in 1999 and again in 2009 at the same clinic in Chongqing, China, showed a two-fold increase in the prevalence of food allergy, from 3.5% to 7.7% ($p = 0.017$), and skin prick tested, SPT, sensitization, from 9.9% to 18.0% ($p = 0.02$). The overall prevalence of challenge-proven food allergy in 0 to 1 year-old children in Chongqing, China was 3.8%, (Chen *et al.*, 2011).

The prevalence rates of adverse food reactions including food allergy were found to be 8.1% (parent-reported) and 4.6% (doctor-diagnosed) in Hong Kong (Leung *et al.*, 2009). The six leading causes of were shellfish (15.8%), egg (9.1%), peanut (8.1%), beef (6.4%), cow's milk (5.7%), and tree nuts (5.0%). When compared with children born and raised in Hong Kong, children born in mainland China had statistically significantly less prevalence. The authors concluded adverse food reactions including food allergy are a common atopic disorder in Hong Kong pre-school children, and prevalence rates are comparable to those in Caucasians. Chan *et al.* summarising what is known about food allergy prevalence in Hong Kong noted 'probable' food allergy in 2010 in children aged 7 – 10 was 2.8% while in 2012 the prevalence of food allergy in children from birth to 14 years old was 4.8% of which shellfish was by far the commonest alongside egg, milk, peanut and fruits (Chan *et al.* 2015b). Children with food allergies have 2 – 4 times higher rates of co-morbid conditions including asthma, rhinoconjunctivitis and eczema. Strikingly Chan *et al.* reported 15.6% of children with food allergies aged 14 years or less are estimated to have a risk of anaphylaxis which is high relative to other countries.

There are over 170 foods known to provoke allergic reactions. Of these, the most common foods responsible for inducing 90% of reported allergic reactions are peanuts, milk, eggs, wheat, nuts (e.g., hazelnuts, walnuts, almonds, cashews, pecans, etc.), soybeans, fish, crustaceans and shellfish (Boye, 2012). However as indicated above there are differences between regions in the patterns of prevalence. Gendel has helpfully collated the way in which different countries legislate for different allergens, (Gendel, 2012).

A large study by the European Food Safety Authority in 2014 recognised the heterogeneity of prevalence studies but suggested that the most common foods triggering about 75% of allergic reactions among children are egg, peanut, cow's milk, fish and various nuts. In adults about half of allergic reactions are caused by fruits of the latex group and of the *Rosaceae* family, vegetables of the *Apiaceae* family, and various nuts and peanuts, (EFSA, 2014a).

5.3.4 Anaphylaxis

Anaphylaxis, a clinical emergency, is an acute, rapid onset, multi-organ systemic allergic reaction with life threatening airway, breathing or circulatory problems. Anaphylaxis can be caused by any allergic reaction and is relatively common with considerable morbidity (Panesar *et al.* 2013; Dhimi *et al.* 2014). First-line treatment of anaphylaxis is rapid intramuscular (into the thigh) adrenaline (epinephrine) typically, in the community, by the person's own autoinjector. Medical aid must be summoned for a range of second-line interventions (Muraro *et al.* 2014a). If the patient is having breathing difficulties, they should be placed in a sitting position, otherwise they should remain lying down in the recovery position with legs elevated. It is crucial that the patient does not stand up as this may result in death from "empty ventricle syndrome".

In children the most common cause of anaphylaxis is food allergy and deaths from food induced anaphylaxis are particularly shocking. Although fatal food anaphylaxis is rarer than accidental death in the general population (Umasunthar *et al.*, 2013) hospital admissions from all causes of anaphylaxis increased by 615% between 1992 and 2012 in the UK. Admission and fatality rates for drug- and insect sting-induced anaphylaxis were highest in the age group 60 years and older. In contrast, admissions because of food-triggered anaphylaxis were most common in teenagers and young adults, with a marked peak in the incidence of fatal food reactions during the second and third decades of life (Turner *et al.*, 2015). It is not possible accurately to predict which allergic individuals are likely to have anaphylactic reactions

however some risk stratification is possible such as coexistent asthma, particularly in children, and a history of previous severe reactions are risk factors. Adolescents are also at a higher risk of anaphylactic reactions owing to biological and social factors. Other factors such as exercise, presence of infection or alcohol consumption at the time of exposure to the allergen can have an influence and there is also a condition recognised as food-associated, exercise-induced anaphylaxis. Swan *et al.* should be consulted for a recent excellent review of the prevention and management of anaphylaxis (Swan *et al.* 2016). The catering sector exhibits particular risks for food related anaphylaxis fatalities, (Leitch *et al.*, 2005 [6] and references therein).

5.3.5 Severity of allergic reaction

From the perspective of possible application of thresholds as a risk management option the most important current issue is that of the severity of adverse reactions, including anaphylaxis. Not only does the threshold dose for symptoms vary between individuals and in the same individual over time but many other factors influence the severity of reaction. Timely, effective treatment limits, but does not control, all reactions and Smith *et al.*, 2015 have reviewed the possible risk factors that prompt a mild or a severe reaction. Fatal and severe reactions appear more likely if there is a combination and alignment of risk factors. For a similar dose in patients with equivalent levels of severe food allergy it is possible to envisage different clinical outcomes. A mild reaction is the outcome in a patient with less current allergic disease, fewer metabolic factors, fewer contributing medications and early effective use of adrenaline / epinephrine and the converse will amplify a severe allergic reaction. The factors include the following and the paper by Smith *et al.* should be consulted for further information on the underlying mechanisms:

- asthma – is probably the most significant risk factor for death from food allergy anaphylaxis and pollen season is also implicated;
- allergic disease burden - severe rhinitis and severe eczema appear to be correlated with increased risk of more serious symptoms in anaphylaxis events;

- intercurrent illness –there is evidence of immunological vulnerability with infective illness;
- comprehension and education – will enhance the prevention, recognition and appropriate and timely therapy of anaphylaxis;
- late or absent treatment – failure or delay in administering adrenaline / epinephrine, is considered to be an important and avoidable factor in fatal reactions;
- medication – Beta-blockers, cox-inhibitors, ACE inhibitors and aspirin have been reported as possible contributors to the severity of all forms of anaphylaxis;
- physiological factors – the expression of multiple allergic mediators (e.g. histamine, interleukins-2, -6 and -10), and serum angiotensin converting enzyme I (ACE) and other enzymes, menstruation;
- the allergen – peanut has been found to cause more severe reactions than other (hazelnut, egg and milk) foods studied;
- concealment of allergen – delayed recognition of an allergic food caused by lipid matrices gives rise to increased dose exposure; itch and burning from spices could mimic allergic symptoms and confuse the issue;
- age – youth is a risk factor for fatal reactions for a variety of reasons including social and emotional while older age has been associated with more severe hypoxemia with anaphylaxis episodes and higher risk of severe cardiovascular symptoms; adults with peanut allergy appear to have more severe reactions than children;
- exercise – can cause anaphylaxis directly and is a co-factor for food anaphylaxis, best defined as food dependent exercise induced anaphylaxis (FDEIA), exercise is also a physiological state that increases release of mediators (e.g. serotonin, bradykinin and endorphins ...);
- alcohol (ethanol) – brings psychosocial and physiological risk factors.

It was also noted there seem to be important co-factors in the community that influence the severity of food allergic reactions outside the controlled clinical setting of a formal food challenge (Smith *et al.*, 2015). A history of severe

allergic events including anaphylaxis has been identified as a risk factor for fatal events but about half of a UK series of food anaphylaxis deaths occurred in patients with a history of mild reactions; thus there can be little reassurance based on a history of previous mild reactions.

At the time of writing the Food Standards Agency funded TRACE Peanut Study was nearing its conclusion. This work is looking at peanut thresholds and how they are affected by two 'extrinsic' factors, exercise and tiredness, known to influence allergen thresholds, (TRACE, 2016).

5.3.6 Quality of Life

Food allergy results in well-documented detriments to the quality of life (QoL), for allergic consumers and their families and carers (King *et al.*, 2009; Venter *et al.*, 2015). Teenagers in particular do not feel that their peers appreciate the difficulties they face and a significant number demonstrate risk-taking behaviour in the management of their food allergies (Monks *et al.*, 2010). DunnGalvin *et al.*, 2015 categorised adverse QoL impacts in terms of social, dietary, and psychological factors. For those living with food allergy social events are experienced differently with feelings of exclusion and difference. Children, teens, and parents need to cope with normal developmental changes (see Hallett *et al.* 2002 for example) as well as with the food allergy, placing them under increased psycho-social stress and leading to adverse effects on QoL and coping. Unsurprisingly parents and carers of food allergic children and teenagers 'live on their nerves'⁴⁶ and find planning for and participation in school, activities and social occasions such as eating out challenging.

To address and attempt to alleviate such stressors, both quantitative and qualitative research suggests that targeting uncertainty should be a major goal for health professionals working with children, teens and families with a food allergy. Remarkable similarities in response to food allergy across countries suggest that policies and programmes that address quality of life

⁴⁶ Gowland, H., personal communication

issues may be relevant to many different populations. An in-depth understanding of the relationship between a diagnosis of food allergy and health-related quality of life, HRQL, as well as the factors that impact it, will ultimately lead to the promotion of earlier, more effective preventive strategies and interventions that are focused on maximising optimal health development and quality of life (DunnGalvin *et al.*, 2015).

Individuals with nut allergies adopt strategies to make safer food choices. Three main such strategies were identified by Barnett *et al.*, 2013 as (1) qualities of product such as the product category or the country of origin, (2) past experience of consuming a food product, and (3) sensory appreciation of risk. Risk reasoning and risk management behaviours were often contingent on the context and other physiological and socio-psychological needs which often competed with risk considerations. Stakeholders could benefit from an understanding of these food choice strategies when risk management policies are designed and developed.

5.3.7 Is There a Cure for Food Allergy?

For those with food allergies lifelong avoidance of the eliciting food(s) is required. Reputed cures for food allergy remain experimental although promising. Small studies have suggested peanut oral immunotherapy (OIT) might be effective in the treatment of peanut allergy. A team in Addenbrooke's Hospital, Cambridge, UK, have established the efficacy of OIT for the desensitisation of children with allergy to peanuts. A randomised controlled crossover trial compared the efficacy of active OIT (using characterised peanut flour; protein doses of 2 – 800 mg/day) with control (peanut avoidance, the present standard of care). OIT successfully induced desensitisation in most children within the study population with peanut allergy of any severity, with a clinically meaningful increase in peanut threshold. Quality of life improved after intervention and there was a good safety profile. Immunological changes corresponded with clinical desensitisation (Anagnostou *et al.*, 2014). These authors recommended further studies in wider populations and that peanut OIT should not be done

in non-specialist settings, but it was effective and well tolerated in the studied age group. For further information see Anagnostou and Clark, 2015.

5.3.8 Prevention of food allergy

Prevention of food allergy has been classified as primary, secondary or tertiary. Primary prevention would block the initial IgE sensitisation, secondary prevention would interrupt the development of food allergy in IgE those sensitised and tertiary prevention would reduce the expression of end-organ allergic disease in patients with established food allergy. A large proportion of the allergy burden is probably inherited. However genetic predisposition alone cannot explain the disturbing increase in food allergy over an evolutionary short 20 year timespan. Studies on changes in gene function in relation to environmental influences (epigenetic modifications) are beginning to provide evidence to explain the mechanisms underlying the development of food allergy. Refer to Du Toit *et al.*, 2016a for further information.

Sensitisation can occur early in infancy, and it appears that prevention strategies should ideally commence during these early-life periods of immunologic vulnerability. Families can be provided with evidence-based advice about preventing food allergy, particularly for infants at high risk for development of allergic disease. The advice for all mothers includes a normal diet without restrictions during pregnancy and lactation. For all infants, exclusive breastfeeding is recommended for at least the first 4 – 6 months of life. If breastfeeding is insufficient or not possible, infants at high-risk can be recommended a hypoallergenic formula with a documented preventive effect for the first 4 months. There is no need to avoid introducing complementary foods beyond 4 months. There is no evidence to support the use of prebiotics or probiotics for food allergy prevention. In 2014, the evidence did not justify recommendations about either withholding or encouraging exposure to potentially allergenic foods after 4 months once weaning has commenced, irrespective of atopic heredity, (Muraro *et al.*, 2014c).

However two studies 'LEAP-On' and 'EAT' reported in early 2016 are important and reassuring additions to our knowledge about possible prevention of food allergy. 'LEAP-On' studied infants at high-risk of developing peanut allergy ('high risk' was defined as infants at with suspected egg allergy based on skin prick testing, and /or with severe eczema based on a clinical evaluation that combined the extent, severity and subjective symptoms of the eczema, and the treatment required).

The earlier Learning Early About Peanut Allergy (LEAP) study from 2015 found, somewhat counter-intuitively, that the majority of such high risk infants can be protected from peanut allergy at age 5 years if they eat peanut frequently, starting within the first 11 months of life. The LEAP-On findings were that early peanut introduction protection is sustained even when peanut is no longer consumed for 12 months.

Enquiring about Tolerance (EAT) in contrast looked at breast fed infants from the general population and the early introduction of six major allergenic foods, peanut, cooked egg, cows' milk, sesame, whitefish and wheat. There were very encouraging findings that peanut and cooked egg allergy in particular and food allergy generally was lower with early introduction. Moreover, although not easy, such introduction was found to be safe.

Taken together these are reassuring findings that may pave the way to stem the epidemic of peanut allergy. These studies were carried out under the close guidance of allergy doctors. Parents should not attempt to replicate what the studies did by themselves but should follow general guidance, for example that encourages mums to breast feed, and common sense attitudes to weaning, introducing a wide variety of foods as appropriate. Parents and carers, especially with infants at high risk, should bring any concerns to their family doctors or other medical advisors for advice (Du Toit *et al.* 2016b; Perkin *et al.* 2016).

At the time of writing the findings of EAT, LEAP and LEAP-On remain to be translated into official guidance and widespread parental practice.

5.3.9 Food allergen management

Businesses too have found the emergence of food allergy challenging. New systems of traceability (Millard *et al.* 2015), management and segregation (Stein, 2015), cleaning (Nikoleiski, 2015), and communication (Flanagan, 2015) have had to be developed. Key industry standards (e.g. BRC, 2015) emphasise greater transparency, traceability and integrity in the supply chain. At the same time incidents and recalls have burgeoned with associated management time, costs and reputational damage (Walker, 2012, [12]). The EU-funded project developing Integrated approaches to food allergen and allergy management (iFAAM), found over 2000 food allergen recalls recorded in the period 2011-2014 based on publicly available information in Europe, North America, Hong Kong, Australia and New Zealand. The biggest incidence of undeclared allergens was found to be for milk and milk products (16 – 31% of all products with recall or alert), followed by cereals containing gluten (9 – 19%), soy (5 – 45%) and egg and egg products (5 – 17%). Between 42 and 90% of the products with recalls/alerts were explained as being 'Not indicated on the label. However, 0 – 17% of products with recalls/alerts were coded as caused by the unintended presence in production of an allergen as the probable result of cross contamination, (known in some parts of the food industry as 'cross-contact') (Bucchini *et al.*, 2016).

It is important to distinguish risk assessment and risk management of food allergy from risk assessment and risk management of food allergens. The former involves patients, families and carers and health care professionals. The latter is a task for all stakeholders, particularly the food industry, regulators, analytical service providers, and food suppliers e.g. caterers, and consumers.

The responsibility for safe and properly labelled food rests with those who make and sell it. The Codex Alimentarius General Standard for the Labelling of Prepackaged Foods harmonises globally the concept of mandatory disclosure on prepacked food labelling of the presence of allergens, with a list of eight major allergens. Gendel has helpfully reviewed country-specific

implementation of Codex requirements on allergens (Gendel, 2012). The food industry seeking to provide safe products, consumer choice and subject to the law must label products accurately and minimise cross-contamination in harvesting, storage, transport, processing of food and cleaning of equipment. The development of 'allergen-free' product lines places a particular burden of responsibility on allergen control. For food businesses there are potentially serious financial impacts and reputational risks of increased food recalls. Compensation in civil law for loss or damage caused by an allergic reaction to a food supplied is a foreseeable risk for food businesses. European food law aims for a high level of protection of human health and consumers' interests. Article 8 of Regulation (EC) No 178/2002 prohibits adulteration of food and fraudulent, deceptive or any other practices which mislead consumers. Article 14 prohibits the sale of unsafe food such as food injurious to health, including the particular health sensitivities of any specific category of consumers (e.g. but not exclusively people with food allergy) where the food is intended for that category of consumers. More specifically, Regulation (EU) No 1169/2011 addresses allergen avoidance risks relating to composition, labelling and food safety. The inclusion in prepacked food of any of 14 major allergens defined by Annex II to Regulation 1169/2011 (replacing Annex IIIa to Directive 2000/13/EC) triggers, with certain limited exemptions, specific labelling requirements extended on 13 December 2014 to non-prepacked food, including catering establishments. Cross-contamination with allergens may trigger general principles of European and UK food law that make it an offence to sell food that is unsafe for, or not of the nature, substance, or quality demanded by, allergic consumers, particularly if specifically intended for their consumption. Hence the food industry must know whether allergens are present in their products and / or production environment and work out ways of controlling them or alerting consumers to the possible risk of their presence through advisory labelling. Allergens in the 'wrong place' can render food unsafe for people with food allergy. The effect of requiring certain allergens to be labelled / highlighted is to prioritise controlling them in the supply chain. However there are many other foods that provoke allergic reactions than are legislated for. Thus foods not listed in legislation as priority allergens must still be managed when known to be allergenic for some people. The UK Food

Standards Agency (FSA) has published comprehensive best practice guidance on allergen cross-contamination and 'may contain' labelling and innovative online food allergy training that is available via <http://www.food.gov.uk/safereating/allergyintol/> . The training includes factory and non-prepacked food scenarios, including in catering, and aims to provide a greater understanding of the issues surrounding enforcing relevant legislation in the area of food allergens for local authority enforcement officers. The online food allergy training course was launched in 2008. The latest FSA guidance was published in August 2014 to help small and medium-sized (SME) businesses comply with new rules on allergen labelling (Gowland and Walker, 2015, [15]). There is an urgent requirement for effective communication between healthcare professionals, patient organizations, food industry representatives and regulators to develop a better approach to protecting consumers with food allergies (Muraro *et al.* 2014b). A framework for categorisation and prioritisation of allergenic foods according to their public health importance has been proposed (Houben *et al.* 2016).

5.3.10 Processing

Food processing has many beneficial effects. However, processing may also alter the allergenic properties of food proteins. It is now well known that roasting increases the allergenicity of peanuts compared to raw. A wide variety of processing methods is available and their use depends largely on the food to be processed. Verhoeckx *et al.*, 2015 reviewed the impact of processing (heat and non-heat treatment) on the allergenic potential of proteins, and on the antigenic (IgG-binding) and allergenic (IgE-binding) properties of proteins. A variety of allergenic foods (peanuts, tree nuts, cows' milk, hens' eggs, soy, wheat and mustard) were reviewed. The overall conclusion was that processing does not completely abolish the allergenic potential of allergens. Currently, only fermentation and hydrolysis may have the potential to reduce allergenicity to such an extent that symptoms will not be elicited, while other methods might be promising but need more data. Literature on the effect of processing on allergenic potential and the ability to induce sensitisation is scarce. This is an important issue since processing

Page 80 of 162

may impact on the ability of proteins to cause the acquisition of allergic sensitisation, and the subject should be a focus of future research. Thus, there remains a need to develop robust and integrated methods for the risk assessment of food allergenicity. (Verhoeckx *et al.* 2015). Processing may also have a profound impact on protein structure influencing its solubility and hence extractability in an analytical process. (Walker *et al.*, 2016)

5.3.11 Precautionary Allergen Labelling

A consequence of the absence of an accepted risk assessment and risk management framework for allergens has been the proliferation of precautionary allergen, 'may contain', labelling (PAL). Wide variation persists in PAL wording, with an estimated 25 different variants of PAL in use, see for example, Hirst, 2014. Within the wide variation there are two principal formats for PAL:

- May contain (X) – this is the simplest format, providing information and with fewer words to take up packaging space,
- Not suitable for people avoiding (X) – the food supplier adopting a more directive approach.

A qualitative study (Barnett *et al.*, 2011) indicated consumers with peanut and/or tree nut allergies adopt a complex range of responses and strategies to interpret PAL. They take into account not only the detail of the labelling but also on external factors such as the nature of the product, the perceived trustworthiness of the producer and the previous experience of the person affected.

Analytical methods for the presence of allergens in food have been used to assess foods on sale carrying PAL to determine the actual presence of unintended allergens. Hirst, 2014, indicated that of foods carrying PAL the total percentage of samples tested in which no allergen was detected was 19% for gluten, 18% for milk, 44% for hazelnut and 45% for peanut. It is therefore understandable that some consumers, basing their decision-making on previous experience may choose to ignore PAL warnings. Thus the

prevalence and variation of precautionary labelling, although intended to assist the consumer in their food choices, is increasingly considered as problematic for food allergic consumers. It is vital that food producers continue to undertake risk assessment for allergen contamination and seek to use clear 'contains' or 'does not contain' labelling wherever possible, using the advice available (Health Canada, 2012; Boye and Godefroy, 2010; FSA, 2006; FSA 2016). It is also clear we need to take into account the rich range of reasoning that consumers draw on to make and justify their decisions to consume products bearing PAL (Barnett, 2013).

It is not surprising therefore that recent global stakeholder reviews view PAL in its current form as counter-productive for consumers with food allergies and call for standardisation of PAL, (DunnGalvin *et al.* 2015; Zurzolo *et al.*, 2016; Turner and Gowland, 2016). Stakeholders agree the lack of agreed reference doses has resulted in inconsistent application of PAL and withdrawal action by enforcement authorities. This has led to a loss of trust in PAL, reducing the ability of consumers with food allergies to make informed choices. The result has been reduced avoidance, reduced quality of life and increased risk-taking by consumers who often ignore PAL. All contributing stakeholders agree that PAL must reflect actual risk. PAL should be transparent and consistent with rules underpinning decision-making process being communicated clearly to all stakeholders. The use of PAL should indicate the possible, unintended presence of an allergen in a consumed portion of a food product at or above any proposed action level. This will require combined work by all stakeholders to ensure everyone understands the approach, and its limitations. Marchisotto *et al.*, 2016 in a study of global perceptions of food allergy thresholds in 16 countries found that understanding of food allergen thresholds and precautionary allergen labelling is limited and consumers may develop their own risk assessment based on labels, which are not based on clinical validation. Improved awareness of thresholds, standardization of PAL, and clinical validation are needed globally. Consumers with food allergy will then need to be advised and empowered to undertake individualised risk assessments in relation to any PAL present.

Before looking at reference doses, action limits and thresholds we should consider some traditional toxicology.

5.3.12 Basic Toxicology

Although for the majority of the population food allergens are not hazards for those with food allergy the allergen to which they are sensitised acts as a toxin when ingested. The assessment and management of the risks that potentially hazardous compounds may pose if present in food are dealt with by the science of toxicology. Examples include food additives, and contaminants including metals, pesticides, veterinary residues and naturally occurring toxins such as mycotoxins (Walker and Wong, 2014, [25]). International and national bodies that deal with food and consumer safety include the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization (FAO), the World Health Organization (WHO), the US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) (EFSA, 2014b). Although the toxicology paradigm has not always been viewed as suitable to deal with allergy various authors have investigated its application to attempt risk assessment and risk management of food allergens (Crevel, 2015). A full treatment of toxicology is beyond the scope of this section however introductions to the subject are available (e.g. ToxLearn, 2015, or a standard text such as Hodgson, 2010). However some discussion of basic concepts may be helpful.

The process of risk assessment is shown in Figure 18 which, since all three are important and inter-related, also includes risk management and risk communication (also see below).

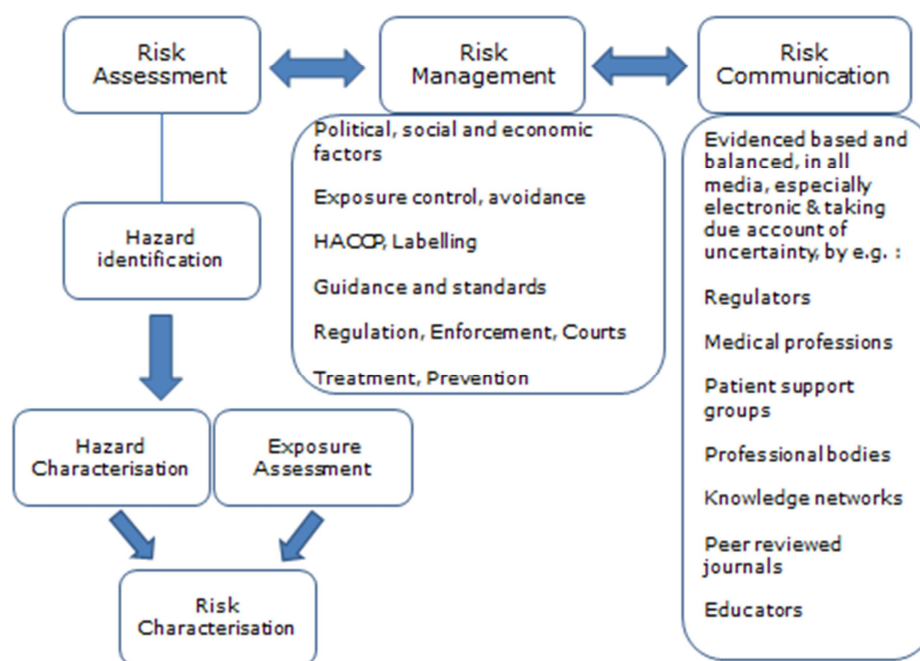


Figure 18: Risk assessment, management and communication

Toxicological risk assessment begins with the identification of the hazard – “the identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system or (sub)-population” (EFSA, 2014b) – usually through epidemiological or animal studies. Food allergy differs in the availability of human clinical data. Recognition of a clinical allergy hazard occurred over 100 years ago (Igea, 2013) but only since the mid 1990’s has food allergy been widely regarded as a public health issue (Crevel, 2015). The *Codex Alimentarius* General Standard for the Labelling of allergens lists eight major allergens of global significance (Codex Alimentarius, 2010) while country-specific variations exist, (Gendel, 2012). The European Union lists the largest number of allergens that are considered sufficiently serious to warrant legislative attention (Table 5). Traditional toxicological exposure assessment attempts to identify potential or completed exposure pathways resulting in contact between the toxin and at-risk populations. It also includes demographic analysis describing the properties and characteristics of at-risk populations that potentiate or mitigate concern and description of the magnitude, duration, and frequency of exposure (Baynes, 2010). Thus, although cumulative exposure appears not to be an issue, many aspects of exposure assessment are problematic for

food allergy, such as prevalence, severity, actual cross-contamination concentrations and unbiased analysis.

Table 5: Food Allergens

Codex Alimentarius¹	European Union²
Cereals containing gluten; i.e., wheat, rye, barley, oats, spelt or their hybridized strains and products of these	Cereals containing gluten, namely: wheat (such as spelt and khorasan wheat), rye, barley, oats or their hybridised strains, and products thereof
Crustacea and products of these	Crustaceans and products thereof
Eggs and egg products	Eggs and products thereof
Fish and fish products	Fish and products thereof
Peanuts, soybeans and products of these	Peanuts and products thereof
Milk and milk products (lactose included)	Soybeans and products thereof
Tree nuts and nut products	Milk and products thereof (including lactose)
Sulphite in concentrations of 10 mg/kg or more	Nuts, namely: almonds (<i>Amygdalus communis</i> L.), hazelnuts (<i>Corylus avellana</i>), walnuts (<i>Juglans regia</i>), cashews (<i>Anacardium occidentale</i>), pecan nuts (<i>Carya illinoensis</i> (Wangenh.) K. Koch), Brazil nuts (<i>Bertholletia excelsa</i>), pistachio nuts (<i>Pistacia vera</i>), macadamia or Queensland nuts (<i>Macadamia ternifolia</i>), and products thereof
	Celery and products thereof
	Mustard and products thereof
	Sesame seeds and products thereof
	Sulphur dioxide and sulphites at concentrations of more than 10 mg/kg or 10 mg/litre in terms of the total SO ₂ which are to be calculated for products as proposed ready for consumption or as reconstituted according to the instructions of the manufacturers
	Lupin and products thereof
	Molluscs and products thereof

1. CODEX STAN 1-1985, General Standard for the Labelling of Prepackaged Foods

2. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011, Annex II. Please see Annex II for certain exceptions to some of the entries.

The reader is advised to consult the latest versions of each in Codex Alimentarius and Eur Lex respectively

Hazard characterisation is “the qualitative and, wherever possible, quantitative description of the inherent properties of an agent or situation having the potential to cause adverse effects” (EFSA, 2014b). Hazard characterization should, where possible, include an assessment of dose-response and an evaluation of uncertainties (WHO, 2009). Dose-response is one of the fundamental concepts in toxicology “...the dose makes the poison...” attributed to Paracelsus (1493 – 1541), (Borzelleca, 2000).

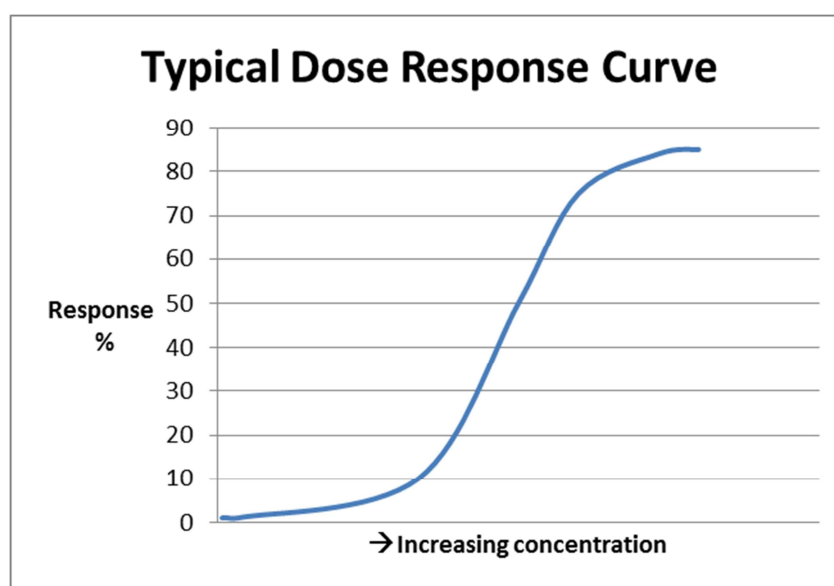


Figure 19: Typical Dose Response Curve

A typical dose-response curve is illustrated in Figure 19, in which the percentage of responding organisms is plotted against the dose or concentration of the compound. The focus of risk assessment is generally on the lower regions of the dose response curve where it is expected that people are realistically exposed. This is often below the experimentally observable range. Chemicals that pose a cancer risk are dealt with differently, see below, but for many chemicals which do not pose a cancer risk there are concentrations below which no response is observed. This is because protective mechanisms are believed to exist that must be overcome before an adverse effect is manifested. The extent to which this is the case for food allergy and the mechanism(s) that underlie any such tolerance are interesting questions. The aim in risk assessment is to identify the upper bound of this tolerance range to obtain a no observable adverse effect level (NOAEL). The NOAEL is the highest dose level that does not produce a

significant elevated increase in an adverse response. Significance refers to biological and statistical criteria and depends on factors such as dose levels tested, number of animals exposed in animal studies, and background incidence in the non-exposed control groups. Sometimes, there is insufficient data to arrive at a NOAEL, and a LOAEL (lowest observed adverse effect level) is derived. The NOAEL is the key datum obtained from the study of the dose – response relationship and is known as the threshold dose. This concept is of significance because it implies that a NOAEL can be used to determine intakes for food additives and contaminants that should be protective of the majority of consumers.

In mainstream toxicology the NOAEL is used to calculate a reference dose (RfD) for chronic oral exposures and, divided by a 'safety factor' or 'uncertainty factor' to calculate acceptable daily intakes, ADI, for food and feed additives and pesticides and the Tolerable Daily Intake, TDI, for contaminants and chemicals in food contact materials. For acute effects, the Acute Reference Dose (ARfD) can also be calculated. The safety / uncertainty factor is often 100 to allow for inter-species and inter-individual variability in toxicokinetics and toxicodynamics.

The RfD is an estimate, with uncertainty spanning perhaps an order of magnitude, of a daily exposure to the human population, including sensitive subgroups that is likely to be without appreciable deleterious effects during a lifetime. The calculated RfD is based on the selected critical study and selected critical end point. The risk assessor may obtain numerous studies where the toxicant may have more than one toxic end point and thus there may be many NOAELs to choose from in the literature. In some instances, even poor data quality may be used to exclude some end points from consideration. Also at issue is determining what is considered an adverse effect, ranging from reversible cellular changes to death. In effect, the RfD is based on the less serious effects rather than serious effects.

Chemicals that are difficult to deal with by traditional toxicology are those that are both genotoxic and carcinogenic where in theory one molecule may initiate a tumour. This tumour initiation may not in practice happen, it is

thought, owing to DNA repair and other protective mechanisms. To address these compounds a 'Margin of Exposure, MOE, approach has been developed. MOE can be used to support prioritisation of risk management action and, if the MOE is very large, communication of a low level of human health concern. However it is essential that the selection of the cancer endpoint and mathematical treatment of the data are clearly described and justified if the results of the MOE approach are to be trusted and of value to risk managers, (Benford *et al.*, 2010).

5.3.13 Allergen Reference Doses, Action Limits and Thresholds

There is a general duty of care on the food industry and obligations in global legislation to reduce and manage the presence of allergens alongside other food hazards. Current evidence appears to enable the establishment of allergen reference doses which might be translated into action limits or population thresholds to underpin reliable food safety management plans for some foods. However, further work is required to include a wider variety of foods and to understand the impact of the food matrix as well as additional factors which affect the progression and severity of symptoms as a function of dose. There is an urgent requirement for effective communication between healthcare professionals, patient organizations, food industry representatives and regulators to develop a better approach to protecting consumers with food allergies (Muraro *et al.* 2014b). Below we examine the development of 'thresholds' and speculate on their future development.

A reminder or introduction of some definitions may assist at this point.

- Threshold – “dose or exposure concentration of an agent below which a stated effect is not observed or expected to occur. It lies in an interval bounded by the LOAEL (upper) and NOAEL (lower)”. Taylor *et al.* 2002) defined the threshold dose as “ ... the lowest amount of the offending food that would elicit mild, objective symptoms (eg, mild urticaria, erythema, and oral angioedema) in the most sensitive individuals.” Thus it is important to note that we are discussing thresholds of elicitation rather than thresholds of sensitisation (Crevel

et al. 2014a). The latter topic is one which is important but much more difficult and outside the scope of this portfolio.

- Reference dose – an estimate of the daily exposure dose that is likely to be without deleterious effect even if continued exposure occurs over a lifetime. In the case of allergens, since acute exposure defines risk for adverse deleterious effect, the exposure estimate is derived from amount per eating occasion.
- Dose distribution – A plot of the cumulative proportion of (allergic) individuals reacting as a function of dose, based on their minimum eliciting doses (Bindsvle-Jensen *et al.* 2002).
- Eliciting dose – the dose (in a dose distribution) which is predicted to provoke reactions in a defined proportion of allergic individuals, commonly stated as the eliciting dose (ED_p) for a percentage of the allergic population p . Thus ED_{50} is the dose of an allergen that will cause a reaction in 50% of the population. ED_5 and ED_1 are the respective eliciting doses that would be expected to be protective of 95 % and 99 % of the allergic population. A ‘minimum eliciting dose’ is the minimum dose that elicits an effect in an individual in a challenge study – equivalent to an individual’s LOAEL.
- Action level – the concentration of an allergen in a product above which some risk management must be carried out, e.g. further efforts to eliminate cross contamination and below which a precautionary label is deemed unnecessary.

In a series of studies Crevel and co-workers have developed the concepts of risk assessment for food allergens that are not used as ingredients in food but arise through cross contamination at harvest, transport, storage or processing. This is also known as ‘cross contact’ or ‘adventitious presence’ but I prefer the term ‘cross contamination’ to connote the unwanted nature of the allergen although the concepts developed to deal with these issues are also applicable to low concentrations of deliberately added ingredients.

Towards the latter part of the 20th century it was questioned if the nature of food allergens precluded risk assessment by classical toxicology such as dose-response relationships. This was challenged by studies by Hourihane

and colleagues working initially on highly refined peanut oil (Hourihane *et al.* 1997a). This was followed by the first study of peanut allergic subjects deliberately to attempt to determine a threshold dose, (Hourihane *et al.* 1997b) and a paper on the threshold concept in food safety and its applicability to food allergy (Hourihane 2001). Hourihane *et al.* administered peanut to 14 subjects in doses from 10 µg to 50 mg, in the form of a commercially available peanut flour. The highest dose of peanut, 50 mg, was well below previous published levels of reactivity (Hourihane *et al.* 1997b). The other innovation was the interspersing of placebo doses between the active doses so that in total 12 active and 12 placebo doses were given in random sequence. This contrasted with previous routine challenge practice of two separate active and placebo challenge series. These authors concluded that even in a group of well-characterized, highly sensitive subjects with peanut allergy, the threshold dose of peanut protein varies. As little as 100 µg of peanut protein provoked symptoms in some subjects with peanut allergy.

Looking back to the introduction to toxicology above we can see that the 'toxicology' of allergens can be described in similar terms. Thus hazard identification occurs retrospectively because individuals are reported to react to a food in a manner consistent with an allergic reaction mediated by IgE. Hazard identification is then ultimately completed by demonstration of IgE binding to individual proteins in the food and confirmatory tests including clinical controlled oral challenges in affected individuals. In this respect allergen hazard identification resembles microbiological hazard identification, which relies principally on epidemiological and surveillance data rather than prospective studies in animals. Hazard characterisation for food allergens thus relies on human data, obviating the uncertainties of animal to human extrapolation of toxicological studies. However, human data also brings ethical and practical constraints in conducting studies that rely on volunteer participants which limits both the amount and type of data that can be generated. Exposure assessment to allergens differs from chemical risk assessment in that it relates to the amount consumed on a single eating occasion, or within a relatively short period of time, rather than long-term exposure; again this resembles microbiological risk assessment.

Nevertheless, the work of Hourihane and colleagues described above paved the way for Taylor and colleagues to ask the question “How much is too much?” Taylor *et al.* described a 1999 roundtable discussion among clinical allergists and other interested parties to share data on threshold doses and to discuss clinical approaches for the acquisition of such data (Taylor *et al.* 2002). It is worth discussing this work in detail because several key concepts were articulated that merit bearing in mind now and for the future.

Although Taylor *et al.* identified considerable clinical data on threshold doses for peanut, cows’ milk, and egg, with limited data for other foods, such as fish and mustard, these data were often obtained by means of different protocols. Hence the estimation of a threshold dose proved difficult and development of a standardised protocol for clinical experiments to allow determination of the threshold dose was recommended. This subsequently was developed (Bindsvlev-Jensen *et al.* 2004).

Taylor *et al.* noted for all practical purposes, allergists had always assumed that the threshold dose for the food to which a patient was allergic was zero and prudently advised patients to adhere to specific avoidance diets. Clinicians thus needed thresholds adequately to advise their patients. Equally, such zero tolerance created enormous practical problems for the food industry, e.g. shared equipment necessitates clean down to prevent cross contamination. This led Taylor and colleagues to a second question: “... how clean is clean enough?”

Taylor *et al.* defined the threshold dose as “... the lowest amount of the offending food that would elicit mild, objective symptoms (eg, mild urticaria, erythema, and oral angioedema) in the most sensitive individuals” (Taylor *et al.* 2002). They also noted the threshold as variable, possibly over an order of magnitude or more between different individuals with the same type of food allergy. Factors contributing to this variability were considered to include exercise, alcohol, and acetylsalicylic acid and the threshold doses for different allergenic foods were recognised as not necessarily equal. Anecdotally, threshold doses were recognised as very small but little or no

quantitative information was available. Presciently Taylor *et al.* attributed paucity of quantitative data to the lack of simple methods for the analysis of the implicated food product for residues of commonly allergenic foods and absence of validated, collaboratively studied, standard methods. The best estimates of the threshold dose for various allergenic foods can be obtained from controlled clinical challenge trials. In only a few cases were such trials intended specifically to determine the threshold dose. More frequently, challenges have been conducted for diagnostic purposes rather than for determining the lowest provoking dose.

Taylor *et al.* listed the lowest provoking doses they had found from the clinical data gathered from DBPCFCs, some single-blind, placebo controlled food challenges (SBPCFCs) and open challenges used for diagnostic purposes. The data were cited as the whole food and in terms of protein. For peanut protein lowest provoking doses ranged from 0.25 mg to 100 mg peanut protein, data for egg protein ranged from 0.13 mg to 200 mg and data for milk spanned 0.6 mg milk protein to 180 mg milk protein. Interestingly data for fish were cited only as the food itself no conversion to protein having proved possible owing to lack of data on the protein content of the fish used. Taylor *et al.* concluded that threshold doses for commonly allergenic foods are finite, measurable, and above zero, however, no attempt to reach consensus on the threshold doses was made at that time. This was owing to the different protocols used to obtain the data but largely because data were mainly LOAELs rather than the more useful NOAELs, the highest dose in the DBPCFCs that did *not* elicit an adverse reaction. The most sensitive patients involved in these challenge trials reacted to the first and lowest dose used. These authors questioned if the acknowledged exclusion of some of the most seriously affected patients (i.e., those with histories of anaphylaxis) from the trials implied that the patients selected for DBPCFC may not be representative of the entire population of individuals with allergies. They speculated if uncertainty factors might need to be applied to NOAELs to determine threshold doses to account for this. The age and body weight of the patients and the nature of the challenge materials were other factors to be considered - standardisation of challenge materials and the vehicles in which they were presented were recommended. Importantly Taylor *et al.*

listed the typical amounts of protein in challenge materials noting conversion between doses expressed as the food and as allergen protein required some important assumptions regarding appropriate conversion factors. For example, the proportion of the major egg allergens Gal d 1 and Gal d 2 as a function of total protein would be higher in egg white than in whole egg. More reassuringly for peanut, little difference appeared to occur in the specific allergen content as a function of variety or agronomic conditions. The conversion data used by Taylor *et al.* included:

- Peanut flour is assumed to contain 50% protein unless the value is specifically known;
- Liquid egg white has an average protein content of 10%;
- Dried egg white has an average protein content of 90%;
- Whole egg has an average of 13% protein on a liquid basis and 50% protein on a dry basis;
- Cows' milk formula is estimated to contain 15 g of milk protein per litre.

The fullest possible reporting of such data and trial conditions (e.g. single or double blind, or open) remain key to current and future derivation of useful threshold data. Taylor *et al.* concluded that the threshold doses for peanut, egg, and cows' milk appeared to be in the low milligram range or higher for most individuals with allergies to those particular foods. Thus these individuals can (and probably do) ingest foods, on occasion, containing lower amounts of their offending food without any untoward reactions. They recommended international efforts to establish threshold doses for commonly allergenic foods using standardized clinical challenge protocols and as wide a range of affected patients as possible.

Much work has been done since the initial investigations of Hourihane, Taylor and colleagues culminating in a series of papers in the first two decades of the 21st century that appear to point the way forward in risk assessment for food allergens.

In a 2007 workshop organized by EuroPrevall, the U.K. Food Standards Agency, and ILSI-Europe, three main, non-mutually exclusive risk assessment approaches were identified (Madsen *et al.* 2009):

- (1) Use of the NOAEL and/or the LOAEL with application of uncertainty factors,
- (2) the Benchmark dose and margin of exposure (MoE) approach, and
- (3) the use of probabilistic models

In the U.S., the Threshold Working Group of the FDA (Threshold Working Group, 2008) also considered multiple approaches:

- (1) defining a limit by statute,
- (2) applying analytical limits of detection (as was done for the sulphites group in the European list of legislated allergens)
- (3) a deterministic approach with uncertainty factors, and
- (4) quantitative approaches including probabilistic modelling.

It is clear that quantitative probabilistic risk assessment provides the strongest scientific approach but is the most data-intensive, with current lack of sufficient data for many allergens and the least transparent to all stakeholders, particularly non-scientists.

Hattersley *et al.*, 2014 reviewed developments in allergen risk assessment, a key paper as the first author was at the time head of the Food Allergy and Food Intolerance team at the UK Food Standards Agency and widely trusted for as a transparent precautionary member of the regulatory community. FSA has maintained a position at the forefront of food allergy research and regulation. Hattersley *et al.* concluded that all stakeholder groups now recognise that zero risk is unrealistic. It is to be noted that this does not necessarily translate to all those with food allergy, or their parents or carers, accepting that zero risk is unrealistic. However Hattersley *et al.* felt it was accepted that classical toxicological assessment and management principles of risks from chemicals or microorganisms in food could be applied to allergens in foods. Crevel and colleagues (Crevel *et al.*, 2014) have described two approaches – ‘deterministic’ and ‘probabilistic’. In the deterministic approach action levels are derived from reference doses, food intake and contamination data by a simple arithmetical method explained below. In the probabilistic approach modelling is used to derive action levels

using food intake and minimum eliciting dose distributions, as well as a certain accepted residual risk level as a starting point.

5.3.14 Deterministic allergen risk assessment

This approach can be used when no or limited data are available on the consumption of the food of interest or its distribution. It is also more practical for the food industry. Action levels can be calculated from an ED value derived from a reference dose and an assumed intake (portion size). This is the approach used by the Allergen Bureau, established on a membership basis in 2005 by the Australian Food and Grocery Council. The Allergen Bureau Food Industry Guide to the Voluntary Incidental Trace allergen Labelling (VITAL) Programme is a standardised allergen risk assessment process for food industry (Taylor *et al.*, 2014; Allen *et al.*, 2014; <http://allergenbureau.net/about-us/>). It is used in Australia and New Zealand but has yet to gain widespread acceptance globally. The VITAL system is free to download and should be consulted in full but operates under the following broad principles:

- Intentionally added allergens must be declared on the product label (e.g. in the List of Ingredients according to local law).
- Action Levels are the concentrations which define the labelling outcomes for each concentration of cross contact allergen. They are determined using the Reference Dose and the Reference Amount/Serving Size.
- Cross contact must be reviewed for opportunities to reduce or eliminate cross contaminant allergens from the product.
- Where cross contaminant allergens cannot be eliminated, they should be labelled as specified by the appropriate Action Level:
- Action Level 1 – precautionary cross contact statement is not required for the relevant allergen under evaluation
- Action Level 2 – precautionary cross contact labelling statement is required for the relevant allergen using the standard VITAL statement.

- Precautionary labelling should only be used after a thorough assessment of the risk. Precautionary cross contact statements must NEVER be used as a substitute for good manufacturing practice (GMP) or as a generic disclaimer. Every attempt must be made to eliminate or minimise cross contact by adhering to GMP
- The ONLY precautionary statement to be used in conjunction with VITAL is: “May be present: name of allergen”

The calculation of action levels is as follows.

$$La = 1000 \times \frac{Rd}{Ar}$$

where

La is the Action Limit above which risk management must take place and below which risk management is less likely to be required; Rd is the reference dose, in milligrams, mg, i.e. the milligram protein level (total protein from an allergenic food) below which according to current data only the most sensitive individuals (between 1% and 5% depending on the quality of the data set available) in the allergic population are likely to experience an adverse reaction, and Ar is the reference amount (in grams, g) – usually defined by manufacturer and the maximum amount of a food eaten in a typical eating occasion. This may be the same as the “serving size”.

A table of reference doses for 12 major allergens can be found in Muraro *et al.*, 2014b, and see also Table 8 in § 8.4.4 below.

As a worked example let us estimate an action level for peanut in a 400 g meal containing meat and 100 g of sauce. Let us suppose there is a risk of peanut flour gaining access to the sauce in the supply chain of the ingredients. How can we use an action level to appraise the results of analytical testing of the product? The data need to use the above equation are:

Rd for peanut is ED₀₁ for peanut protein of 0.2 mg; Ar is 100 g

$$\text{Thus } La = 1000 \times \frac{0.2}{100} = 2 \text{ mg kg}^{-1} \text{ peanut protein.}$$

That is to say, a concentration of more than 2 mg kg⁻¹ (ppm) peanut protein in the sauce is a risk for at least 1 % of the peanut allergic population and risk management measures are required. The ‘dilution’ of the sauce by the meat, which could be separately tested and assessed may give a margin of error but bear in mind the uncertainty in the ability to measure peanut protein in the sauce may approach ± 50%.

Does this mean that if we find less than 2 mg kg⁻¹ (ppm) peanut protein in the sauce the meal is safe for peanut allergic consumers? This is not so easy a question to answer, especially if the inadvertent presence of peanut is not homogenous – particulate peanut fragments rather than peanut flour.

In practice, the food industry may be nervous of such an approach. An ED₀₁ has an underlying risk that 1 in 100 allergic individuals will have a reaction; is this an acceptable balance of risk? It may be acceptable to a food business selling 1,000 units a week, but not to a food business selling 100,000 units a week. Food retailers may be tempted to, and probably do, opt for the analytical limit of detection as a default action limit, which may not bear any relation to true risk. Thus we need to factor in sales and consumption as a measure of exposure, and the percentage of the population who have the allergy (Points, 2016).

5.3.15 Probabilistic allergen risk assessment

Some of the above questions may be addressed by the probabilistic approach. Spanjersberg *et al.*, 2007 developed a quantitative risk assessment model for allergens based on probabilistic techniques and presented a case study, hazelnut proteins in chocolate spread.

Kruizinga, *et al.* performed a sensitivity analysis on a previously developed probabilistic model to predict the likelihood of an allergic reaction due to unintended exposure to food allergens to identify which parts of the model most influence the output (Kruizinga, *et al.* 2008). The model included the proportion of the population which is allergic, the proportion consuming the

food and the amount consumed, the likelihood of the food containing an adventitious allergen and its concentration, and the minimum eliciting dose distribution for the allergen. A shift in the distribution of the minimum eliciting dose reflecting a more potent allergen, and an increase in the proportion of the population consuming a food, increased the number of estimated allergic reactions considerably. In contrast, the number of estimated allergic reactions hardly changed when the minimum eliciting doses were based on a more severe response, or when the amount of food consumed was increased.

Spanjersberg *et al.*, 2010 prompted by a severe allergic reaction in a cow's milk protein allergic patient to a dark chocolate product containing undeclared milk protein applied probabilistic modelling to investigate to what extent allergen concentrations of unlabelled products reach levels that are of public health relevance. The concentrations of milk proteins in the complaint sample and a collection of products of other batches and brands purchased from different stores were determined. Together with appropriate threshold and food consumption data, the risks of allergic reactions and the severity of these reactions within the adult milk-allergic population were determined using probabilistic risk assessment techniques. The results showed that milk protein concentrations in unlabelled products reach levels that may elicit allergic reactions in up to 68% of the adult milk allergic consumers.

Rimbaud *et al.* 2010 reported a quantified risk assessment of the consumption of peanut in chocolate products. The occurrence of adventitious peanut protein in chocolate and the dose-response relationship were estimated with a Bayesian approach using available published data. The consumption pattern was described by a French individual consumption survey. Risk simulations were performed using second-order Monte Carlo simulations, which separately propagated variability and uncertainty of the model input variables. Peanut allergens were found to occur in approximately 36% of the chocolates, leading to a mean exposure level of 0.2 mg of peanut protein per eating occasion. The estimated risk of reaction averaged 0.57% per eating occasion for peanut-allergic adults. The 95% values of the risk were between 0 and 3.61%, which illustrates the risk variability. The conclusion was that adventitious peanut allergens induce a risk of reaction

for a part of the French peanut-allergic population. The method was considered to be capable of generalised development to assess the risk due to the consumption of every foodstuff potentially contaminated by allergens.

Rimbaud *et al.*, 2013 revisited this topic. Food products analysed for the possible presence of peanut traces in scientific literature were selected. For each foodstuff, the allergic risk associated with their consumption was estimated using the French individual food consumption survey, representative of the general French population. An internet survey on the attitudes of peanut-allergic individuals toward food precautionary labelling was conducted. For three foodstuffs, the allergic risk was then refined integrating the information on specific food behaviours of French allergic individuals. Considering the mean probability, inadvertent presence of peanuts was identified in 20% to 37% of products. Adults were exposed to up to 12.5 mg of peanut protein on 97.5% of their eating occasions. The mean risk of reaction ranged from 0.2 % to 2.4%. Considering eating occasions for all the products, 1.5% of the peanut-allergic adults would have at least one allergic reaction in a week. This demonstrated the benefits of integrating all available information to underpin decision making in the area of food allergen cross-contamination and highlighted the need to generate more data to further refine the risk assessment for the benefit of allergic consumers.

Crevel *et al.* 2014a reviewing the development of risk assessment for food allergens noted dose distribution modelling of minimum eliciting doses permitted the quantification of the risk of reaction at the population level and has been readily integrated with consumption and contamination data through probabilistic risk assessment approaches to generate quantitative risk predictions (Crevel *et al.* 2014a). These authors discuss the strengths and limitations of this approach and identify important data gaps, which affect the outcomes of these predictions. These include consumption patterns among allergic individuals, analytical techniques and their application, severity-dose relationships, and the impact of extraneous factors which alter an individual's physiology, such as infection or exercise. Nevertheless, Crevel *et al.* conclude application of these models has provided valuable insights, leading to further refinements and generating testable hypotheses.

Crevel *et al.* also identified challenges relevant to each component of the risk analysis: risk assessment (data gaps and output interpretation); risk management (clear and realistic objectives); and risk communication (clear articulation of risk and benefit) (Crevel *et al.* 2014b). It was noted that translation of the outputs from risk assessment models into risk management measures must be informed by a clear understanding of the model outputs and their limitations. Crevel *et al.* considered this would lead to feasible and achievable risk management objectives, grounded in a level of risk accepted by the different stakeholders, thereby avoiding potential unintended detrimental consequences. Clear, consistent and trustworthy communications actively involving all stakeholders were recognised as necessary to underpin these objectives. The conclusions, integrating the perspectives of different stakeholders, offer a vision where clear, science-based benchmarks form the basis of allergen management and labelling, cutting through the current confusion and uncertainty. Finally, these authors recognised that the proposed framework must be adaptable to new and emerging evidence.

Crevel *et al.* have given a comprehensive analysis of the research and knowledge gaps of both the deterministic and probabilistic approaches to quantitative allergen risk assessment (Crevel *et al.* 2014a). Deterministic allergen risk assessment is already carried out however given the considerable resource implications it is unlikely that the food industry will routinely adopt probabilistic allergen risk assessment in the near future.

However, if, as is currently the case, different measurement approaches give different results, sometimes markedly so, for the same sample, and results cannot be anchored by reference materials, it will be impossible to make use of thresholds properly. The way forward is described in the concluding section of this volume.

6 Food Authenticity – Discussion

6.1 Introduction

That food accurately matches its description or labelling (food authenticity) has been increasingly important to consumers for many years and hence to the agrifood sector. Its converse - mislabelling or misdescription or food fraud (when misdescription is carried out for financial gain), is detrimental to both consumers and legitimate trade sectors. Establishing authenticity and detecting fraud are underpinned by a range of activities including enforcement sampling and analysis. We have discussed in section 3.2.1 the distinction between food safety and food authenticity. However in many cases they overlap – e.g. replacement of almonds with peanuts, an allergy risk that as we have seen (section 5.2.2) that can prove fatal. There is also the well recorded morbidity and mortality from counterfeit alcoholic drinks (e.g. Arslan *et al.*, 2015). Food authenticity and food fraud are, though, mainly a value for money issue and one with which regulators and Public Analysts were concerned from the 19th century onwards. Appraising the nature or substance or quality of food was and is a constant feature of enforcement science and selling, to the purchaser's prejudice, food which is not of the nature or substance or quality demanded is an offence under Section 14 of the Food Safety Act 1990. The outputs discussed here range from the routine chemical assessment of the honesty of quantitative declarations on food labels to the molecular biology of the horse meat episode that made global headlines for over 6 months in 2013.

6.2 QUID - Quantitative Ingredients Declaration

As the number of compositional standards in food law (prescribing the amount of meat in product, for example) were reduced in the 1980's to allow more flexibility in new product development and greater nutritional variety, the *quid pro quo* for consumers was 'QUID' Quantitative Ingredients Declaration. These provisions require the quantity of an ingredient or category of ingredients used in a food to be indicated on the label when (a) that ingredient or category of ingredients appears in the name of the food or is usually associated with that name by the consumer; (b) that ingredient or

category of ingredients is emphasised on the label in words pictures or graphics or (c) that ingredient or category of ingredients is essential to characterise a food and to distinguish it from products with which it might be confused because of its name or its appearance. The current EU legal basis for QUID is in Article 9(1)(d), Article 22 and Annex VIII of Regulation (EU) No 1169/2011. A food manufacturer is able to determine the numerical values for a QUID declaration from the recipe weight (mass balance) of ingoing ingredients and there is a well-established body of guidance on this. However checking QUID declarations for enforcement purposes requires an analytical approach to estimate the quantity of declared, often high value ingredients (e.g. meat and fish) by analysis of the end product. Some examples will help to make the requirements clear, Table 6⁴⁷ in which I have also indicated the analytical approach that could be used to verify the QUID declaration.

One of the most profitable frauds in mass produced compound products is to sell water at the price of meat and fish and analytical end product policing of this relies heavily on nitrogen factors.

Table 6: Examples of QUID

Product	Possible Product description	QUID declaration required for	Analytical approach
“Lancashire hot pot”	mutton and potatoes with onions, carrots and gravy	mutton	Nitrogen factor / DNA PCR
“chilli con carne”	minced beef with kidney beans, tomatoes, peppers, onion and chilli	minced beef	Nitrogen factor / DNA PCR
“fisherman's pie”	cod and haddock with peas in a white sauce, topped with mashed potato	fish	Nitrogen factor
“summer pudding”	strawberries, raspberries, blackberries, redcurrants and blackcurrants set in a light gel with bread	fruit	Potassium, and/or anthocyanin content

⁴⁷ Adapted from: The Food Labelling Regulations 1996: Guidance Notes on Quantitative Ingredient Declarations (“QUID”), FSA

<http://www.food.gov.uk/sites/default/files/multimedia/pdfs/quid.pdf> (accessed 03.08.2016)

6.2.1 Nitrogen factors – a critical review

The approach that has stood the test of time for flesh foods (meat, fish and shellfish) is that originally demonstrated by Stubbs and More (1919) working in the Laboratory of the Government Chemist. Recognising the characterising component of meat and fish is its protein, and hence nitrogen content, the method is based on the determination of the nitrogen content of a sample and its comparison, corrected for non-flesh nitrogen, with the species specific nitrogen concentration, the 'Nitrogen Factor'. In modern routine analysis moisture, fat, mineral matter and hydroxyproline are also determined; the latter to estimate the amount of connective tissue. There are now well established procedures for the calculation of 'defatted meat', a hypothetical intermediate datum from which 'lean meat' and 'total meat' can be estimated. There are legislative limits for the amount of fat and connective tissue that are allowed to be included in the ingredient declarations of beef, pork and chicken etc. and these limits must be taken into account in the determination and calculation of meat content. Nitrogen factors for meat species (but not white fish) are expressed on a fat-free basis, N_{ff} , following the example of Stubbs and More, as this approach simplifies subsequent calculations. The caveat 'apparent' is prefixed to reported data if non-meat nitrogen (other than in certain rusk fillers) has not been accounted for. Standard well known works give a fuller treatment of the procedure ('Pearson' 1991; McLean 2007) and there are also examples in our 'salmon' paper, [17].

There are of course limitations in the use of nitrogen factors. They are average values, and when deciding whether declarations of meat or fish content are accurate, it is important to bear in mind the possible variability of natural values and the analytical variability of their determination. Pre-packed fish products may still use the generic ingredient description "fish" or give the type of fish used, and in either case the percentage of these ingredients present in the product. It is not possible accurately to assay 'QUID' in products containing mixed species of meat or of fish using nitrogen factors, nor products containing mixtures of meat and offal. Thus in assessing compliance against a QUID declaration, if an apparent deficiency is revealed by end-product analysis, it has always been Walker's practice to suggest an

in-factory investigation, with the benefit of recipe information, trade data and official sampling and analysis of the ingredients. At the same time a series of observations should be obtained which if necessary may be used either to provide advice to the manufacturer or in a subsequent prosecution. For example, three separate formal samples taken at appropriate intervals of time, say, a month apart, or three separate production batches, should be analysed to build up an official data set. The nitrogen content of a food can be determined using a standard method whose performance characteristics are well documented, either Kjeldahl (1883) or Dumas (Burns 1993) both well characterised in the literature as standard methods. The two methods are not quite equivalent, the Dumas method was found to provide results that are higher than that of the Kjeldahl method, by about 1.4% of the mean Kjeldahl nitrogen. The generally accepted explanation is that any non-protein forms of nitrogen present in samples are converted into elemental nitrogen in the Dumas method.

However difficulties can arise in the interpretation of the nitrogen content of a sample in the absence of a validated set of data for the appropriate species nitrogen factor. Such a situation arose in toward the end of the first decade of the 21st century when it was represented to the Government Chemist by both trade and enforcement sources that a robust modern nitrogen factor dataset representative of modern farmed Atlantic salmon was required as the 1973 nitrogen factor of 3.60 (based on wild salmon) was suspected of leading to artificially low calculated fish contents. In addition there was no published basis for the use of a nitrogen factor of 3.60 for salmon frame mince, a by-product of salmon fillet processing. Hence the Government Chemist initiated a study to provide a validated database of variations of nitrogen factors for farmed Atlantic salmon and salmon frame mince from a range of locations and sampled at different times of the year. In the course of preparing the study report for publication Walker and Burns both realised that no review had been carried out of extant nitrogen factor data, which were often challenged by the trade. Walker and Burns and colleagues set about remedying the want of a single convenient overview, clarifying that validated databases for nitrogen factors for meat, poultry or fish are, by definition, those published in peer reviewed journals. These authors also confirmed that

the only extant data was that produced in association with the Association of Public Analysts (formerly the Society of Public Analysts) or by the Analytical Methods Committee (AMC) of the Analytical Division of the Royal Society of Chemistry (formerly the Society for Analytical Chemistry). The review [16] covered the period from 1919 to 2010 and, recognising the costly nature of nitrogen factor studies made recommendations for their more cost effective derivation from properly attested industry sources. Data on nitrogen factors continues to be gathered and published by AMC Nitrogen Factors Sub-committee⁴⁸ currently ably chaired by Dr Mark Woolfe. The data are published open access both as AMC Technical Briefs⁴⁹ and in the RSC journal *Analytical Methods*.

6.2.2 Nitrogen factors for farmed salmon and salmon frame mince

The reason for the work on farmed salmon is given above (§6.2.1). The study was a lengthy and interesting one involving a large team from the Government Chemist Programme. Walker was involved in planning the study, the factory visit to inspect the process and gather the samples, training laboratory staff to properly fillet the whole salmon and evaluating and writing up the results. Key statistical input was by Steve Ellison. The results demonstrated that the nitrogen factor of 3.60 previously in use based on a 1973 study, is no longer appropriate for farmed Atlantic salmon. The species nitrogen concentration of farmed Atlantic salmon is lower than that reported in 1973 for fish in the wild. Our data on fat-free nitrogen content was consistent with the known relative stability of nitrogen and variability of the fat in smolts during the on-growing phase of their development at sea prior to harvest. Therefore Nitrogen factors on a fat free basis, Nff, were recommended to be used for the estimation of the amount of salmon (*Salmo salar*) in compound products. Where the country of origin (Scotland or Norway) and product type are known Table 7 in the published paper may be consulted for a specific Dumas Nff failing which a general Nff for Dumas N

⁴⁸ RSC AMC Nitrogen Factors Sub-committee (accessed 03.08.2016)

<http://www.rsc.org/Membership/Networking/InterestGroups/Analytical/AMC/NitrogenFactors.asp>

⁴⁹ RSC AMC Technical Briefs (accessed 03.08.2016)

<http://www.rsc.org/Membership/Networking/InterestGroups/Analytical/AMC/TechnicalBriefs.asp>

determinations of 3.80 (3.75 Kjeldahl) is suggested for salmon (*Salmo salar*) flesh processed as described in Appendix 2 of the paper.

After the salmon is machine filleted valuable flesh remains on the 'skeleton' or 'frame'. Salmon frame mince is produced after washing salmon skeletons in an air agitated container of potable water to reduce the microbiological load of the produced mince. The washed skeletons are then fed into a 'Baader' machine. This is a revolving drum with 3 millimetre holes through which flesh from the skeleton is pressed by pressure from a belt, (Figure 20). For *Salmo salar* frame mince a general Dumas factor, Nff for of 2.85 (2.81 Kjeldahl) is suggested. It was further suggested that salmon frame mince is materially different from salmon and must therefore be separately identified in the list of ingredients of compound products in which it is incorporated. Anomalous analytical findings against QUID declarations of salmon content must be followed up by in-factory investigation. Further work on the quantitative estimation of salmon and non-salmon lipids in compound products is required.



Figure 20: The production of salmon frame mince

6.3 Forensic molecular biology

Despite what will be discussed in the next section on the horse meat episode the UK Government has had a > 20 year world leading programme on food authenticity, initially in the former MAFF⁵⁰, from 2000 to 2010 in FSA and now in Defra⁵¹. The food authenticity programme has developed many novel analytical authenticity approaches including high resolution NMR, carbon isotope ratio analysis and DNA techniques. Walker chaired a quinquennial review of the programme in 2004.

The flexibility, relatively lower costs and probative value of DNA methods render them particularly effective. However their deployment in the forensic environment of UK Official Food Control Laboratories (OCLs, Public Analysts) staffed mainly by analytical chemists, required knowledge transfer of molecular biology techniques. This transfer of DNA methods was carried out during the tenure of FSA of the Food Authenticity Programme mainly under its then Head Dr Mark Woolfe. After formal retirement Dr Woolfe has remained professionally active and was recruited in 2010 by Walker to join him in a successful bid for a small project let by Defra to assess the effectiveness of the knowledge transfer and uptake of DNA methods by Public Analysts. The main challenge of the work was to persuade the Public Analysts to discuss frankly their experiences and it is, in all conscience, doubtful if another team would have been as trusted as Walker and Woolfe in that regard.

A structured approach was taken to obtaining the required information. A detailed questionnaire was prepared, intended to elicit information from the participants on all aspects of the transfer of DNA methods and their application. It was specifically designed to look at the ability to use the methods by participation in proficiency testing schemes as well as the number of samples or studies the methods had been used in. Relevant laboratories and personnel were identified, contacted to elicit their cooperation and the questionnaire sent by e-mail. Completion of the questionnaires involved holding face to face interviews, either at the

⁵⁰ Ministry of Agriculture Fisheries and Food

⁵¹ Department for Environment Food & Rural Affairs

respondent's laboratory or a convenient central meeting place, failing that by telephone and e-mail. In addition, interviews were also held with 2 contractors, who had been involved with developing the DNA methods and running the training courses and challenge tests. Visiting the laboratories was one of the most enjoyable aspects of the work as many of the scientists were known to Walker but an opportunity to visit their laboratories had never before presented itself.

The assessment findings highlighted that the knowledge transfer was well planned and highly effective with the main objective of embedding a suite of DNA methods in 11 out of 13 eligible laboratories, thus increasing capability in food forensic molecular biology to (11/13) 85 %. The transfer of 5 DNA methods (fish species, meat and exotic meat species, bushmeat species, Basmati rice, and orange juice adulteration with mandarin juice) gave Public Analysts an increased range of effectiveness with fish species identification having been particularly successfully applied and resulting in successful prosecutions of fraudulent activity. Given the financial constraints in UK Public Analysts a beneficial outcome was a strategic refocussing of effort boosting enthusiasm and excitement for food authenticity issues. A further outcome of the transfer and evidence of the uptake of DNA technology was the adoption of Real Time Polymerase Chain Reaction techniques by a critical mass of OCLs, permitting advanced application to problematic authenticity issues such as the detection of adulteration of durum wheat pasta with common wheat, detection of meat ingredients in vegetarian foods, and the quantitative determination of GMOs in single ingredient foods such as pasta, rice and soya. Other recommendations arising out of the study were to adapt, to a lab-on-a-chip platform, DNA methods for pig and cattle breed authentication including wild boar, and an improved Basmati rice authentication. Finally, it was recommended that sustainable deployment of DNA methods to address food authenticity and fraud hinges on regulatory salience of the need for it and this, along with future priorities, should be kept under review.

The paper [18] and full report to Defra [19] informed the strategic priorities of the Food Authenticity Programme in a valuable manner and it is fair to say

that had the DNA method transfer not taken place the UK would have been in a much worse position to deal analytically with the subsequent horse meat scandal than it in fact was. In retrospect however, and in subsequent discussions with the Food Authenticity Programme's independent Programme Advisor, Walker came to realise that the report was deficient in one key aspect. The phrase "sustainable deployment of DNA methods to address food authenticity and fraud hinges on regulatory salience of the need for it" was code for "use it or lose it". The sustainability of the Public Analysts and deployment of forensic molecular biology in their hands was much more precarious than the report disclosed. Walker felt at the time that demonstrating the evident usefulness of the techniques and their successful adoption by the Public Analysts, the only scientists by training and practice competent to present the results in court, was the best way to secure sustainable funding. That turned out not to be the case and it would have been better to be frank that the funding framework was not in place in a sustainable manner to guarantee indigenous UK food forensic enforcement molecular biology to a acceptable level.

6.4 Horse meat 2013

On 15 January 2013 the Food Safety Authority of Ireland, FSAI, published a press release on a small survey identifying horse and pig DNA in burger products, initiating a meat substitution scandal that involved most of Europe and maintained high and lengthy media and political salience. Walker saw the press release almost as soon as it was published and grasping its significance was as well prepared as most for the media storm that followed. Walker recorded an interview on the horsemeat issue for Sky News, broadcast 16th January and appeared briefly in an episode of the BBC 1 Food Inspectors on 23 January. Also on the horsemeat issue he was featured live on Sky News on 30 January and again in a recorded interview on BBC Newsnight the same evening. On 11 February his recorded interview on phenylbutazone was aired on BBC 10 O'Clock News and on 11 February he did a telephone interview with BBC Radio Ulster and took part in a

background briefing to journalists at the Science Media Centre⁵², London. Thereafter Walker's media work in this area continued at a reduced pace. The SMC briefing led to contributions to a number of print media stories including in the Guardian, the Economist, the Irish Times, City AM and international coverage via Associated Press. On 26 Feb Michael spoke at the All Party Group at the Northern Ireland Assembly and was an invited speaker at a SMC event at Queen's University on 20th March, with an interview on Radio 5 Live on 23 March. A quote appeared in the Independent on Sunday as late as 02 June 2013. Throughout the episode Walker sought to be reassuring where safety was not compromised, candid where knowledge was lacking on the reasons behind the episode and robust in condemnation of food fraud. His independent calm, scientific voice was acknowledged by the SMC as valuable⁵³.

The Government Chemist Programme was soon involved as challenges to Public Analysts findings of horse meat in various beef products arose. Within LGC separate teams were mobilised to deal with the referee cases and, recognising LGC's long established multidisciplinary expertise and acknowledged culture in regulation, accreditation, policy and standard setting, to advise Defra, FSA and the analytical community as the episode progressed. Walker led the referee analysis team.

On 9 April 2013 a Government Chemist supporting statement was issued on the horse meat referee cases mainly authored by Walker and approved by the Government Chemist, Dr Derek Craston.

We can confirm that the Government Chemist was contacted - as the Government's independent referee - to advise on samples

⁵² The Science Media Centre housed in the Wellcome Collection, aims to provide, for the benefit of the public and policymakers, accurate and evidence-based information about science and engineering through the media, particularly on controversial and headline news stories when most confusion and misinformation occurs, <http://www.sciencemediacentre.org/about-us/> (accessed 03.08.2016)

⁵³ For an internal review an independent consultant approached stakeholders for comment and obtained the following: "LGC's Michael Walker took part in our emergency press briefing in February 2013, which resulted in at least 30 pieces of coverage. The speed and scale of media coverage was a recipe for a potential health scare and the appetite for comments and opinions could have resulted in the public hearing only from non-experts or campaigners with an agenda. LGC's willingness to speak to journalists helped ensure that coverage was more accurate and evidence-based, and that the public heard from the best experts. Michael entirely accepted the need for scientists to share their expertise even when that feels risky and uncomfortable. We have cited his courage as an example when trying to recruit similar experts." – Dr Fiona Lethbridge, Senior Press Officer, Science Media Centre

related to suspected horsemeat or pork presence in beef product samples. Five samples of beef products were referred to the Government Chemist in March 2013. For three of the samples the Government Chemist was asked to determine if horse meat was present and for two samples to determine if pork was present.

As an independent referee, we are not able to comment on individual cases beyond saying that the Government Chemist's findings confirmed those of the Public Analysts, in that either horse or pork was found in the relevant samples. The Government Chemist has sent individual findings, in the form of an official certificate, to each Local Authority that referred a sample, requesting the Local Authority to pass it on to the food businesses concerned. The Government Chemist has also sent all the findings to the Food Standards Agency. DNA meat speciation analysis provides sensitive and specific tests for the presence of horse DNA in beef products. By concurrent analysis of gravimetric mixtures of horse flesh in beef it is possible to form an opinion on the response from PCR amplification of nuclear DNA in relation to the equivalent of 1 % w/w of raw horse in raw beef. A quantitative DNA/DNA approach is the subject of current validation work.

As the crisis abated Walker decided to draft a review of the episode, enlisting the help of the senior molecular biologist in LGC Dr Malcolm Burns and Professor Duncan Thorburn Burns. The review was published [20] on 13th January 2014. The review summarised the extent of the substitution, placing it in an historical, food authenticity, food safety and analytical context and drew conclusions on the future measures recommended primarily to government. It concluded that history teaches us that this will happen again but not in quite the same way. Walker and co-authors suggested it is unlikely that widespread horse meat substitution will reoccur for decades but other frauds will arise and the way to guard against this is continued systematic vigilance. The challenge is to secure a cost effective, efficient scientific infrastructure to support that vigilance in a planned and sustainable manner. This latter comment was supportive of recommendations Walker had made as a Subject Matter Expert to the Elliott Review.

Walker found that the UK Parliamentary record appears to be the sole continuous public record in which periodic concerns about horse meat are extant. He found horse meat concerns expressed in 1886, the Sale of Horseflesh &c Regulation Act 1889, in debates around the adoption of the

Food and Drugs Act 1938, and again in 1941 and 1943. There were references in the 1950's and the provisions of the 1889 Sale of Horseflesh Act were essentially retained in the Food and Drugs Act 1955 and the Food Act 1984. In the meantime, the “great meat substitution scandal” unfolded in Australia in 1981 that rivalled in extent the 2013 scandal and was documented in a Royal Commission Report of which only a small fraction is readily available. In 1991 a Tribunal of Inquiry into the Beef Processing Industry (the “Beef Tribunal”) was set up in the Republic of Ireland.

The review paper also charts the history of the UK Food Authenticity Programme noting that it was very active in meat speciation research and surveys between 1998 and 2010 although no surveys that included horse meat were undertaken from 2003 until after the FSAI press release of January 2013. The review documented *in extenso* the horse meat episode, critiqued methods of analysis, described the referee cases, discussed the application of food law to the issue and summarised the UK and EU responses to the crisis.

In the meantime, the Elliott Review of which Walker was a member published its interim report in December 2013. The final report was to follow published in December 2014. The concluding section of this volume will discuss aspects of the Elliott Review in greater detail.

6.5 Lessons from the past – butter and margarine

The final paper in this section [21], prompted by the way in which horse meat fraud periodically reoccurred, dealt with the history of food adulteration and fraud and attempts at their control from the middle ages to date in Belgium and the UK with special reference to butter and margarine. The development of analytical procedures for the authentication of milk fat were outlined, from those based on the characterisation fatty acids derived from milk fat in the nineteenth century to chromatographic methods in the next century and recent rapid spectroscopic approaches. The importance of adequate surveillance programmes to reduce the incidence of food fraud was again stressed.

7 Chemico-legal practice

7.1 Introduction

As noted above food law in the UK is largely criminal law and Public Analysts from their inception as consulting chemists have always been forensically aware with both civil and criminal cases in their general practice. Walker is no exception. The outputs in this section illustrate his chemico-legal practice insofar as it has been able to put a fraction of it in the public domain.

7.2 Food irradiation

Paper [22] describes a case of two food samples, chilli powder, that were alleged to have been irradiated, contrary to European and UK Law. Walker was asked to revise a draft of the paper, based on the case, which had stalled in the submission process owing to adverse comments by the reviewers. Walker was able to redraft the paper to address the reviewers' objections and the paper was accepted. The study dealt with the outsourcing of the confirmatory method of detection of irradiation which requires the use of irradiation facilities, which LGC does not possess. The the work was organised to ensure that the evidence was provided by expert laboratories in such a way that it was fit for use in court. In particular, aspects of quality assurance essential to the detection of irradiation in blends were described. Analysis was carried out using two standard techniques; photostimulated luminescence and thermoluminescence in each of two laboratories. Both reported the referee samples as positive and correctly identified irradiation in blends of irradiated and non-irradiated chilli powder, at concentrations as low as 1% irradiated material. A second case, consisting of a sample of Guarana powder, was submitted shortly after the first and was treated in a similar manner. On the basis of the result reported by the Government Chemist, the owner of this sample accepted a formal caution from the food authority and paid the prosecution costs.

7.3 Review of referee cases

As an integral part of the governance of the Government Chemist Programme regular updates on the conduct and outcomes of referee cases are given to the advisory committee that oversees the work. For many years a conference has been held publically to disseminate the lessons learned from referee cases. The conference has grown to a two day event held every two years but always features referee outcomes as one of the talks. A permanent record in general terms is also available in the annual Government Chemist review. Paper [23] was drafted to explain in more detail the conduct and outcomes of referee cases in a given period of time. The paper discusses the legal framework for referee cases and casework in the calendar years 2010 and 2011. The paper provided an opportunity to assess the performance of the technical appeal safeguard and the control system in the limited number of complex cases where appeal has been invoked. Walker and Gray⁵⁴ noted the OCL system in the UK faced continuing funding challenges in 2010 and 2011 but in general performed well in areas where capability has been developed such as in aflatoxin analysis where Public Analysts' and Agricultural Analysts' findings were confirmed on technical appeal in 5 out of 7 (71.4 %) cases. However much more dispersion was evident in aflatoxin results between laboratories in animal feed samples than in food samples. Since largely the same laboratories are involved it is clear that sampling, and in particular the lack of a requirement for high shear mixing with water to form a slurry prior to splitting the samples into parts, is the main source of the variation. It was recommended that sampling and sample preparation should be harmonised in the feed and food areas. This now appears to be the practice evidenced by more recent cases.

OCL performance was less good in the more problematic area of drug residue analysis. Of six nitrofurán marker metabolite cases (all on imported crustaceans) only one (17 %) was completely upheld. Research published in 2011 demonstrated that the marker for nitrofurazone, semicarbazide SEM, is naturally occurring in crustacean shells and in two cases (33 %) the

⁵⁴ Kirstin Gray project manages the laboratory aspects of each referee case and in many instances carries out laboratory work on the samples. Walker is glad of this opportunity to acknowledge her commitment and practical expertise.

Government Chemist confirmed SEM not detected in the core flesh of the animals overturning the OCL findings. Walker published an advice note on nitrofurans analysis⁵⁵ and since then the number of disputes has diminished.

7.4 Alcohol back calculations

By virtue of the MChemA qualification Walker is an authorised analyst under Article 19(6)(a) of the Road Traffic Offenders (NI) Order 1996 and equivalent legislation in Great Britain and appears in the annual RSC list of Road Traffic Acts Analysts⁵⁶, a copy of which must be given to any defendant who is likely to have a sample of blood or urine analysed for alcohol. Samples in this regard form a small element of his chemico-legal practice as do theoretical calculations for forensic purposes of systemic alcohol concentration at a time other than when a sample is taken for measurement of its alcohol concentration, commonly called 'back calculations'. There are several reasons for adducing such evidence at trial, either to establish the systemic alcohol concentration at the time of driving if there was a long delay in obtaining a sample, or to propose that drinking after driving (the 'hip flask defence') explains the blood breath or urine alcohol concentration found. The 'hip flask defence' is allowed in Northern Ireland by reason of the Court of Appeal decision of 22 October 1993 given by Hutton LCJ and Kelly LJ in the case of *King and Carson*.

In 2013, in order to inform the legal profession in Northern Ireland of the basis of such calculations, and the information that should be furnished to a practitioner when the calculation is requested, Walker and Burns offered a paper to 'The Writ', the Journal of the Law Society of Northern Ireland. The paper was accepted, published in two parts [24].

⁵⁵ Followed by a full paper not included in this portfolio - John Points, D. Thorburn Burns, Michael J. Walker, 2014, Forensic issues in the analysis of trace nitrofurans veterinary residues in food of animal origin, *Food Control*, 50, 92-103

⁵⁶ Royal Society of Chemistry, RSC, list of Road Traffic Acts Analysts
<http://www.rsc.org/globalassets/13-help-legal/help/road-traffic-analysts-booklet.pdf>
(accessed 04.08.2016)

7.5 Protection of the Agri-Food Chain by Chemical Analysis: The European Context

This and the following output are chapters in a book, 'Practical Food Safety: Contemporary Issues and Future Directions', published in May 2014. Both were written with a colleague, Yiu-Chung Wong, in the Government Laboratory in Hong Kong. Each author respectively took the lead in drafting each chapter.

One chapter, [25], describes the objectives and basis of European food and feed law and discussed how new food and feed law is developed in the EU and how it works in day-to-day regulation in the United Kingdom. This is illustrated with EU legislation and typical analysis for: (1) contaminants, for example, the genotoxic carcinogenic fungal metabolites, aflatoxins and other mycotoxins; (2) veterinary residues, for example, carcinogenic nitrofurans; and (3) aluminium in imported noodles. The chapter describes the context (potential impact on consumers), the law and the nature of the chemical analysis undertaken to protect consumers and responsible traders. Finally, it focuses on situations where disagreements arise between laboratories acting for the regulator and for the trader and how these are resolved in the UK.

7.6 Achieving Quality Chemical Measurements in Foods

The final output in the portfolio, [26], describes international harmonization of quality assurance protocols for laboratories to ensure appropriate quality control of measurements such as for food ingredients, nutrients, contaminants and additives. The milestone in standardization of internationally accepted good laboratory practice is ISO/IEC17025 - General requirements for the competence of testing and calibration laboratories. This standard document highlights method validation, measurement uncertainty (MU) and various quality control procedures such as the essential requirements for laboratories to produce consistently valid results in chemical testing and calibration. In addition, it describes metrology in chemistry (MiC) a discipline concerning accurate chemical measurement through the implementation of traceability, comparability and MU. The establishment of a national MiC framework from recognizable reference standards or systems

provides a crucial foundation to promote global recognition of analytical results. Standardization of laboratory quality assurance protocols and application of MiC are considered to be vital to ensure reliable analytical results in food measurement.

The co-location within LGC of the Government Chemist Programme and the National Measurement Institute for chemical and bioanalytical measurements, the embodiment of UK metrology in chemistry, and both headed by the Government Chemist is a synergy that has many beneficial outcomes, not least the wide expertise available to Walker in conducting referee cases. It is the basis of LGC's long established multidisciplinary expertise and acknowledged culture in regulation, accreditation, policy and standard setting mentioned in section 6.4.

The cooperation with the Government Laboratory in Hong Kong⁵⁷, dates back to 1879, and which serves as the Public Analyst Laboratory, the Hong Kong National Measurement Institute for chemistry and the Forensic Science Laboratory, has proved enduring and fruitful for LGC corporately and for Walker and his colleagues professionally and personally.

⁵⁷ Government Laboratory of the Government of the Hong Kong Special Administration Region, <http://www.govtlab.gov.hk/> (accessed 04.08.2016)

8 Conclusions

8.1 Introduction

Compiling and reflecting on this portfolio of work over the past few years guided by Professor Declan Naughton⁵⁸ has been a valuable opportunity to reassess a professional career that, while stressful and demanding at times, has been enjoyable and productive.

There are several outcomes that are considered of note illustrated in Figure 21.

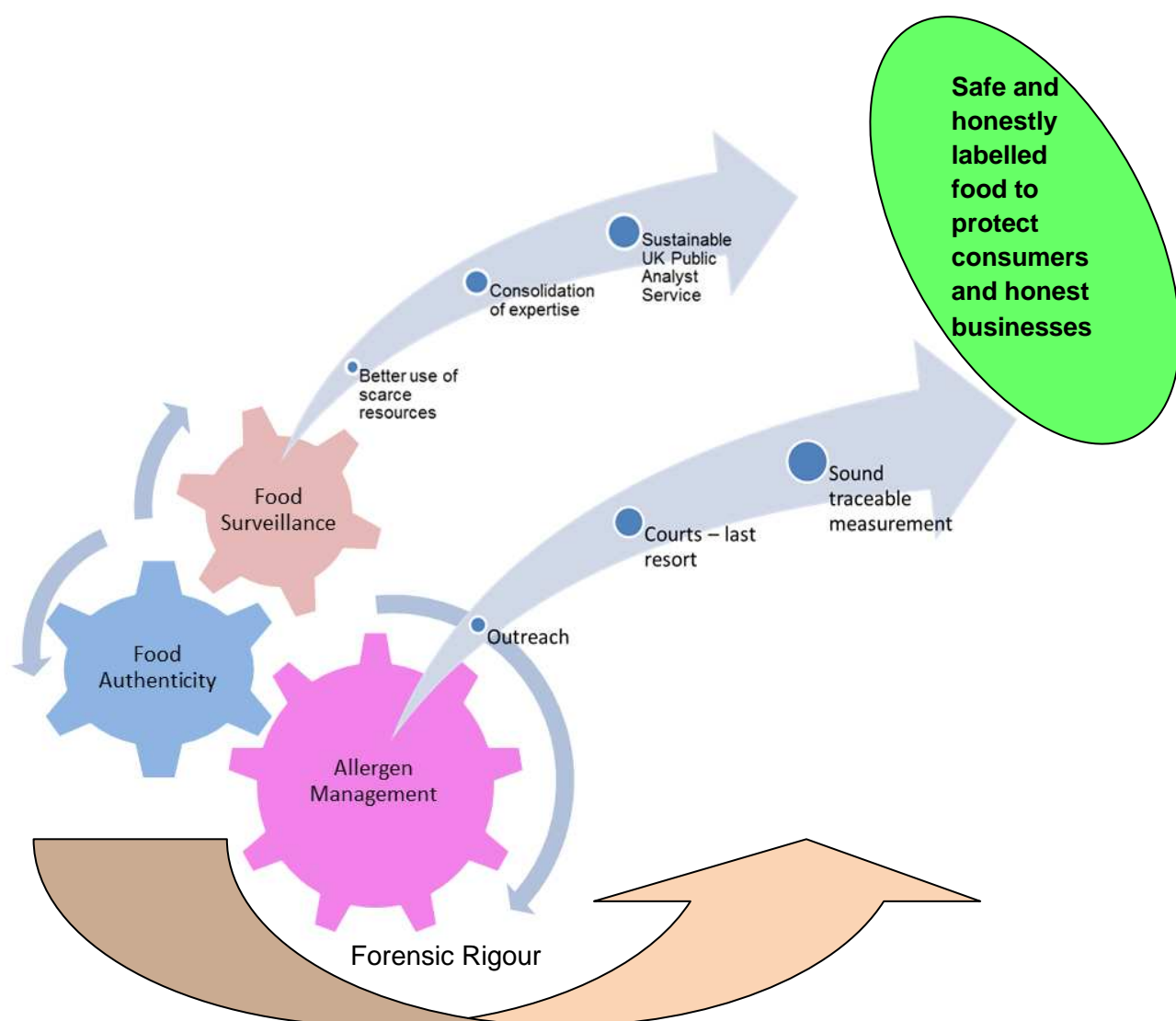


Figure 21: Suggested outcomes of Walker's work

⁵⁸ And less formally by Professor Duncan Thorburn Burns
Page 118 of 162

Permitted food additives, allergens and food fraud pose minimal risks for the majority of consumers. Yet many evince concern about them and all pose real risks, additives and allergens for significant minority population groups and food fraud when it jeopardises safety. Equally, all pose quality of life, financial or resource risks for consumers, honest businesses, regulators and health care services.

The scientific investigation of food additives and food authenticity began in the 19th century and are well understood and regulated within mature disciplines. By contrast, food allergy was a clinical rarity in the 1980s and limited regulation of allergens in food began only in 2003.

There are facets that unite these disparate topics and similar solutions to the different problems they pose.

8.2 Food Surveillance

Regulation and enforcement of legal requirements for food additives by the application of analytical chemistry was a routine matter in both jurisdictions on the island of Ireland early in Walker's career. What was lacking was practical cooperation between the jurisdictions and collation of the results of analysis beyond immediate decisions as to compliance. Walker's work with Galway Public Analyst Laboratory and publication of baseline data on food additives (§ 4 above) fostered practical cooperation and foreshadowed the work of the FSA in NI⁵⁹ Strategic Committee on Food Surveillance, FSANI-SCFS, now chaired by Walker. Under Walker's guidance FSANI-SCFS grew to include not only EHOs and Public Analysts but officials from the NI Department of Agriculture, Environment and Rural Affairs, DAERA, formerly DARD, and scientists from the Agri-Food & Biosciences Institute, AFBI. The FSANI-SCFS annual reports thus now cover a broader spectrum of work and give a more holistic picture of protection of food safety and standards in Northern Ireland. FSANI-SCFS circulates and promotes strategic priorities for

⁵⁹ FSA in NI – Food Standards Agency in Northern Ireland
Page 119 of 162

sampling and analysis to brigade the efforts of the 11 local authorities in NI. This is self-evidently more efficient in times of ever decreasing resources. Moreover, stimulated by the interaction between measurement science and policy e.g. in his work on morpholine, dimethyl yellow and jelly mini-cups Walker helped FSANI-SCFS to look upon sampling and analysis in a more productive way. FSANI-SCFS priorities now set out the *Problem to be Addressed*, the *Public Benefit* from sampling and analysis and the *Expected Outcome*.

Examples from the 2014 report in Table 7 illustrate this approach in moving away from sampling and analysis of what was ‘always sampled and analysed’ to focus on work planned to demonstrate benefits for broader society. Walker’s plans for FSANI-SCFS include links with epidemiologists in Queen’s University to take advantage of biobank data further to plan societally more beneficial food surveillance.

Table 7: Chemical Priorities (FSANI-SCFS)

Priority Target	The Problem	The Public Benefit	The Expected Outcome
Contaminants - mycotoxins	Mycotoxins are cancer causing chemical that occur naturally in food from mould growth if the food is badly stored	The public are protected from high amounts of these dangerous chemicals in food	Contributes to fewer cancers in the population
Allergens	For those with allergies the presence of unexpected allergens in food can cause illness of even death	Food fraud that allowed peanuts into the food chain detected and prevented. Awareness of new rules on allergen labelling assessed	Life threatening food fraud prevented. Better quality of life for people with allergies as new labelling rules adhered to
Food authenticity - counterfeit spirits - Horse meat and other species /offal substitution	Food fraud harms economically, reduces consumer confidence and allows criminals to infiltrate our food supply network.	Consumers are not ‘duped’ and get what they expect, and pay for. Dangerous counterfeits (e.g. toxic spirit drinks) kept out of food chain	Another crisis in consumer confidence is averted; criminals deterred from interfering with our food supply chain

8.3 Food Authenticity – the Elliott Review

Species substitution was always a potential problem well-recognised by Public Analysts but regulation was caught unawares by the horse meat scandal of 2013. Walker traces this in part to the reduction in horse meat surveillance after 2003 and the Elliott Review paints a broader picture of the improvements needed to avert a similar scandal in the future.

Walker's work described in sections 6 and 7 above prepared him for his input to 'Elliott'. The chapter in the final report of the Elliott Review that Walker was most intimately involved in drafting was Chapter 5 on Laboratory Services. As the work of French and Phillips and of Rothstein show the evolution of regulation of food safety and authenticity is a complex process involving many actors in civil society. The science community often are unaware of the subtleties. It was this realisation and the still fragmentary nature of local government (Oddy 2007 § 3.2.1 above) that influenced Walker as part of Professor Elliott's review to propose the amalgamation of the remaining six local government owned Public Analyst Laboratories in England and the further amalgamation of this new shared service with Public Health England. This was in addition to the more immediate problems around methods discussed in detail in Chapter 5 of 'Elliott'.

Three key outcomes flowed directly from Walker's work with Professor Elliott. These are (a) the Virtual Authenticity Network, (b) recognition of centres of expertise in authenticity research and (c) a shared, merged public sector Public Analyst Laboratory Service. Figure 22 shows Walker and Elliott's vision for the inter-relations that should pertain, and which have largely now developed. At the Government Chemist Conference on 22 June 2016, Jon Griffin, President of the Association of Public Analysts described the work underway to create the Association of Local Authority Public Analyst Laboratories, ALAPAL. The formation of this entity, bringing together the remaining six local government owned Public Analyst Laboratories in England was formally announced at RSC headquarters Burlington House on 19th of July 2016. There remains much to be done but these developments are a promising sign of progress. In particular the consolidation of expertise

within a larger ALAPAL will permit the specialisation and multidisciplinary teams that are needed to address 21st century problems in food surveillance.

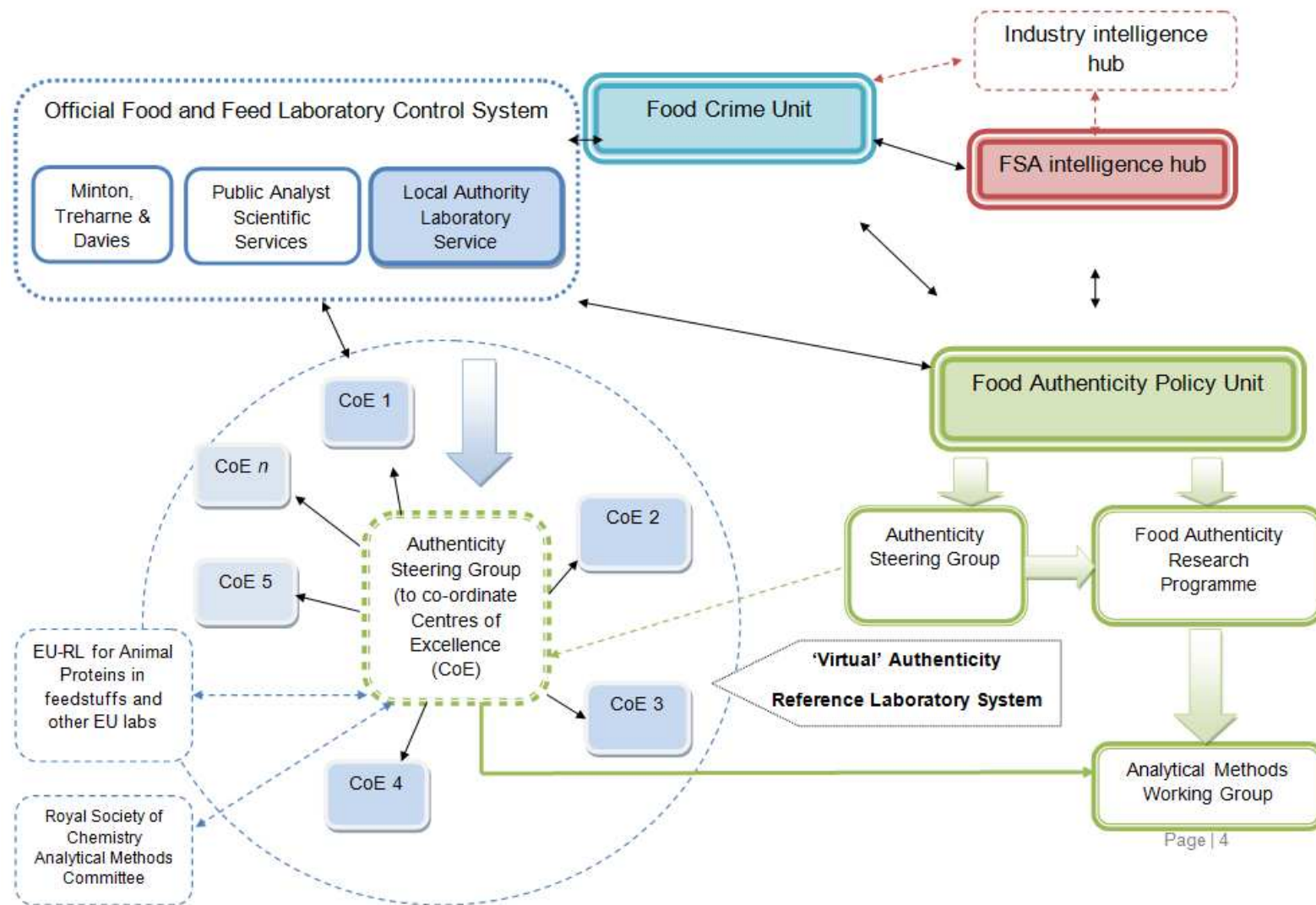


Figure 22: Vision in the Elliott Review of official inter-relations to safeguard food

8.4 Food Allergens

The least tractable problem Walker has grappled with remains very much a work in progress. There remain empirical difficulties in the risk assessment of food allergy grounded in knowledge gaps of its toxicogenomics, (§ 5.3.15). Moreover problems associated with the analysis of allergens in general subvert the application of deterministic or probabilistic risk assessment and management of food allergens.⁶⁰

Two incidents illustrate well the vulnerability of the food supply chain, and hence of allergic consumers. These examples (a) illustrate evidence of deliberate substitution of almond by peanut in the supply chain and (b) describe what was initially thought to be deliberate adulteration of cumin with almond but in fact turned out to be contamination of the cumin supply chain with mahaleb. Both examples demonstrate that good allergen analysis is necessary to help protect the food supply chain.

8.4.1 Almond or Peanut?

As we have seen above enforcement surveillance of allergen compliance in catering establishments regularly concludes that specifically asking for an allergen-free meal provides little real protection. Such appeared to be the case for two chicken tikka masala meals found to contain peanut in a survey in 2010/11. However, follow up revealed a supplier had introduced groundnuts (peanuts) instead of almond powder as contracted into the supply chain. The firm was convicted on prosecution. The conviction was, however, overturned on appeal on technical legal grounds. Nevertheless the Food Standards Agency (FSA) Annual Report of Incidents 2012 refers to investigations of severe allergic reactions following the consumption of curry dishes purchased from Indian restaurants and takeaways. Noting that some of these incidents resulted in fatalities, FSA reported that some incidents were caused by the use of a ground almond ingredient, which also contained

⁶⁰ The material that follows was drafted by Walker and appears in Walker, M.J., Burns, D.T., Elliott, C.T., Gowland, M.H. and Mills, E.C., 2016. Is food allergen analysis flawed? Health and supply chain risks and a proposed framework to address urgent analytical needs, *Analyst*, 141(1), pp.24-35 wherein references may be obtained.

ground peanut (groundnut). FSA identified weaknesses in the food chain where such contamination and loss of clear information occurred, including poor understanding of the significance of substituting peanuts for almonds, incorrect allergen information provided at a point of sale, and unclear labelling and confusion between peanuts and tree nuts (almonds) leading to the potential for accidental substitution. However FSA also reported possible economically motivated adulteration, driven by the financial incentive to substitute ground almonds with ground peanut and as we have also seen above a conviction for manslaughter has resulted from just those circumstances.

8.4.2 Almond or mahaleb?

Against this backdrop in October 2014 when Canadian authorities found undeclared peanut and almond protein in products containing cumin, it was feared that a further, potentially life threatening, breach of supply chain security had occurred. The FSA issued the first of a small number of related recalls, of ground cumin sold by the Barts Ingredients Company Ltd found to contain traces of almond protein not listed on the label, on 31 January 2015. FSA referred this as an official technical appeal to the Government Chemist, asking for a review of the analysis that had led to the recall. In early March 2015 Barts Ingredients Company Ltd claimed publically that another material, mahaleb, gives a positive reading for almond using test methods. On 30 April 2015 the Canadian authorities rescinded product recalls of cumin and cumin-containing products previously thought to contain undeclared almond. The Canadian statement noted that the recalls had been based on “original laboratory results [that] were false positives ... [caused by] cross-reactivity of mahaleb ... (*Prunus mahaleb*), with the almond allergen test kit. It is highly likely that the positive sample results for the ground cumin and cumin-containing products were due to mahaleb contamination and not almond”. Almond is a member of the genus ‘*Prunus*’ - trees and shrubs, which includes plums, cherries, peaches, nectarines, apricots and mahaleb. *Prunus mahaleb* was previously little known in the UK but was said also to have been handled in the cumin supply chain. The UK Government Chemist subsequently determined that although limitations still remain in the state of the science that

prevent the presence of almond being completely ruled out, the results of the technical appeal investigation indicate that the queried sample contained a *Prunus* protein and DNA the origin of which was consistent with *mahaleb* rather than almond while *P. mahaleb* does not appear in the list of allergens required by law to be declared if used intentionally as an ingredient in food, it is important for a food business to understand its supply chain to assess and manage cross contamination risks. Given the amino acid homology between almond and mahaleb a risk to *Prunus*-allergic individuals might remain.

8.4.3 Allergen analysis

Analysis for food allergens is required for many reasons. Key industry standards emphasise greater transparency, traceability and integrity in the supply chain requiring analysis to check that food is what it is claimed to be, and encourage systems to reduce exposure to fraud. Analysis supports validation and verification of factory cleaning and investigation of recalls and incidents. Surveillance and enforcement, particularly after the introduction of more extensive labelling requirements, rely heavily on analysis to support and protect consumers and responsible businesses and, in the event of adulteration, provide evidence for criminal or civil action in the courts; a key deterrent. Investigation of adverse reactions may require analysis to find out what caused the reaction, and therefore enable the individual to avoid it in the future. Investigation of fatalities, already problematic, requires analysis e.g. of food seized at the incident, stomach contents or other forensic exhibits.

The rescindment, (see above) because of initially flawed analysis, of allergen recalls on both sides of the Atlantic risks uncertainty and confusion over allergen testing in the future. This jeopardises consumer safety now and the development of allergen thresholds in the future. The origins and resolution of these problems lie in the difficulties of allergen analysis.

Food allergens that bind to IgE are large protein molecules and many approaches have been taken to their analysis. As shown above most routine food allergen analysis is undertaken by Enzyme Linked Immunosorbent Assay (ELISA) enabling detection and (semi-) quantification. Polymerase

Chain Reaction (PCR) assays are also applied in allergen risk assessment and management. For both techniques detection is less of an issue, although not without problems but sound quantification remains elusive. Commercially available ELISA kits exhibit variable and manufacturer specific sensitivities (§ 5.1.4 above) and cross-reactivity. Recent work on precautionary labelling on pre-packed processed food and concentrations of certain cross contaminant allergens in foods suffered from unexpected cross reactivity in the commercial ELISA. False positive results were identified arising from an Association of Analytical Communities (AOAC) approved peanut assay owing to cross reactivity to soya. The cross reactivity which was evidently not a feature of the original assay seemed to have developed after many years use of the kit and necessitated a troublesome late stage review of the research findings. Structural changes in the target molecules by food processing or sample extraction may prevent detection. PCR assays are probative of the source species DNA (which may not be present e.g. egg white) rather than the allergen protein. Moreover proteins are the hazard and thus the key measurand. PCR is essentially qualitative at present. Quantification based on copy number can be derived from cycle thresholds but requires reference materials to construct a calibration curve, although digital PCR may circumvent this difficulty. Even so is not easy to convert a quantification based on copy number to a weight/weight basis. There has been little systematic research on the relationships between the findings of PCR approaches and protein techniques such as ELISA or liquid chromatography and tandem mass spectrometry, LC-MS/MS, for allergen analysis to assess their comparability. Hence, there is a requirement for orthogonal methods that confirm molecular identity and that are capable of valid quantification. LC-MS/MS methods, e.g. multiple reaction monitoring of peptides arising from enzymatic digestion of proteins, offer such advantages, along with the possibility of multiplexed high throughput. The application of LC-MS/MS is still recent in food allergen analysis. It is possible to detect proteins and peptides with a high degree of sensitivity and resolving power, providing protein composition, structure and sequence information, and MS has the potential for a wide linear dynamic range, and absolute identification and quantification of allergens. However the techniques require a high level of expertise and costly equipment; extraction and cleanup steps are necessary

and the methods can be laborious and time consuming. The complexity of most food matrixes represents a significant challenge even to MS although guidance is available, including [13] on a model system that demonstrates isotope dilution mass spectrometric traceability from a set of peptides to an allergenic protein. Because ELISA is much more widely used for allergen analysis than MS or PCR there is more published evidence of its deficiencies but similar deficiencies apply to both MS and PCR approaches. Thus the promise of MS or PCR will be lost if underpinning work is not carried out.

In summary, current allergen analysis would be impossible without ELISA which has brought many benefits in allergen risk assessment and risk management. However all current forms of allergen analysis present some deficiencies which may jeopardise present and future risk assessment and risk management of food allergy, a problem of high and increasing importance

8.4.4 A framework to address the problems

Three distinct but interrelated areas of analytical work are urgently needed to address the substantial gaps identified (1) development of reference methods resulting in metrologically traceable results, (2) production of reference materials, and (3) a bioinformatics gap analysis. The first two will bring allergen analysis into line with the analysis of additives in food while the third is required to deal with previously unrecognised contaminants such as mahaleb.

8.4.5 Reference materials

Sykes *et al.* 2012 showed that inclusion of a 'reference spiked sample' in a Proficiency Test, PT, round where the raw data were non-normal and multi-modal, tended to yield ratio data that were normal and symmetrically distributed. Many have called for the development of internationally recognised sets of allergen reference materials to improve the reliability of allergen analysis. Reference material (RM) and Certified Reference Material (CRM) are well defined terms within an associated international infrastructure.

It is not always clear that the limited number of food allergen RMs currently commercially available comply with this infrastructure. Reference materials produced by National Measurement Institutes exhibit the highest standards.

Making a reference material is relatively expensive owing to the complexity of production. The following steps should be carried out within a documented quality system:

- *Effective project planning and project management*
- *Definition of need, background and clear specification*
- *Material procurement*
- *Identification or development of a validated analytical method for the measurand so as to distinguish measurement dispersion from dispersion arising from homogeneity and stability issues*
- *Preparation of the material by well characterised methods*
- *Packaging to ensure integrity and stability*
- *Storage under controlled conditions to maintain stability*
- *Homogeneity and stability evaluation by validated methods with known performance data*
- *Characterisation and certification (if that proves possible) with documented traceability of values, an uncertainty budget and consideration of the commutability of the material*
- *Preparation of a certificate to accompany the material, and a production report*
- *Distribution and sales, ensuring integrity of the material*
- *On-going monitoring and customer support.*

Thus producers of allergen RMs should address the above points and attempt to ensure the matrix is industrially realistic for processed food. The incurred concentrations should be appropriate for and preferably establish a relationship with the concentrations that affect allergy sufferers. A prototype such material set, (a blank material and a QC material with peanut protein added at 10 mg kg⁻¹) has been prepared by Holcombe and Walker (§ 5.1.6) based on a EuroPrevall study matrix (chocolate dessert mix) used to assess clinical thresholds.

Both calibrant and matrix reference materials for food allergens are required. However their production is not trivial. The legislation defines allergens in terms of the food but analysis targets proteins or their peptides (or DNA). For the protein allergens the analyte is often neither exactly defined nor easy to render identical in sample and calibrator. Typically, multiple allergen proteins and isoforms are present, in a complex matrix. Taking peanut as an example, the food itself includes proteins, lipids, carbohydrates and minerals available in multiple processed formats including raw, roasted and / or defatted to varying degrees and included in a wide range of other foods. The peanut allergens include at least 12 - 14 multiple specific proteins, of which only Ara h 1, Ara h 2, Ara h 3, and Ara h 6 have been demonstrated to be clinically important. Protein post-translational modifications, PTM, occur and further complexity is introduced by biological variation, fractionation (intended and adventitious) and reaction with other food components. The analyte (measurand) therefore may be, in MS, peptides expected to be uniquely representative of specific proteins, for ELISA, known proteins that may or may not be the allergens or, for PCR, a DNA sequence. Pragmatism is required as ideal solutions to the above problems will not easily, economically or soon be found. Therefore a staged approach is needed starting with non-ideal reference materials, as explained below. Maximum transparency is required as to the commercial origin and compositional characteristics of the allergenic food used to formulate, initially, simple matrix reference materials gravimetrically prepared at blank (zero) and clinically relevant allergen concentrations. Such concentrations are suggested in Table 8. Homogeneity and stability studies and further characterisation by, at least, ELISA should be performed. Experience gained will enable progression to incurred allergens in processed foods representing a suitable spectrum of protein, lipid and carbohydrate compositions, followed by production of certified reference materials representing those RMs found most useful. It should be noted that production of reference materials is rarely a commercial proposition.

Table 8: EAACI Reference doses and suggested clinically relevant reference material concentrations

Food	EAACI Reference Dose Muraro <i>et al.</i> , 2014	Suggested clinically relevant [§] RM allergen protein concentrations mg kg ⁻¹
Peanut ED 1 %	0.2 mg peanut protein	2 - 10
Cow's milk ED 1 %	0.1 mg milk protein	1 - 10
Egg ED 1 %	0.03 mg egg protein	0.3 - 5
Hazelnut ED 1 %	0.1 mg hazelnut protein	1 - 10
Soya ED 5 %	1.0 mg soya protein	10 - 100
Wheat ED 5 %	1.0 mg wheat protein	10 - 100
Cashew ED 5 %	2.0 mg cashew protein	20 - 100
Mustard ED 5 %	0.05 mg mustard protein	0.5 - 5
Lupin ED 5 %	4.0 mg lupin protein	40 - 200
Sesame seed ED 5 %	0.2 mg sesame protein	2 - 10
Shrimp ED 5 %	10 mg shrimp protein	100 - 1000
Fish ED 5 %	0.1 mg fish protein*	1 - 10

ED x %, Eliciting Dose for x % of the allergic population

* provisional

§ assuming a minimum portion size of 100 g

Much can be learned from work that led to what remains the 'gold standard' reference material for gliadin, described in the proceedings of the Working Group on Prolamin Analysis and Toxicity (WGPAT). Securing food that is free from gluten (gliadin) for those with coeliac disease is as important as an allergen-free diet and fraught with the same analytical difficulties (see [9] and § 5.1.3 above). Moreover the definition of gluten is empirical: "gluten" is defined as a protein fraction from wheat, rye, barley, oats or their crossbred varieties and derivatives, to which some persons are intolerant and that is insoluble in water and 0.5M NaCl. The prolamin content of gluten is generally taken as 50% and prolamins are defined as the fraction from gluten that can be extracted by 40 - 70% ethanol. This definition enabled WGPAT to prepare a gliadin reference material by extraction from milled wheat kernels representing a specific year's harvest of the most commonly grown cultivars

in 3 European countries. Moreover the obtained gliadin (PWG-gliadin) was characterised by a wide spectrum of techniques including immunological, MS and electrophoretic as well as for stability and homogeneity, as summarised by van Eckert *et al.* In 2005 the Institute for Reference Material and Measurements of the European Commission (IRMM) declined to accept PWG-gliadin as a certified reference material and returned it to WGPAT in 2006 from where it can be obtained. Although it is difficult to speculate on the reasons for IRMM's action, the want of a route to full metrological traceability for PWG-gliadin, (and allergens in general), that the proposals below seek to address may be one of the root causes.

It is recommended that a coordinated international programme be set up for the production of properly characterised reference materials and calibrants for allergen analysis, beginning with the rapid availability of simple materials (e.g. containing, separately, the major allergens (e.g. as defatted and/or freeze dried powdered substances) at zero (blank) and clinically relevant concentrations (Table 8). The programme should progress to incurred allergens in processed foods representing a suitable spectrum of protein, lipid and carbohydrate compositions, followed by production of certified reference materials representing those RMs found most useful.

8.4.6 Metrologically traceable methods

Metrological traceability is the property of an analytical result that allows measurements made in different laboratories under different conditions to be compared in a meaningful way, within an international infrastructure, the International System of Units (the SI). Such work is carried out by National Measurement Institutes (NMIs), in each developed country. Metrological traceability of allergen protein data is currently possible only by MS-based absolute quantification such as isotope dilution MS, IDMS, a primary ratio method that relates results directly to the SI with a small measurement uncertainty, and which is commonly used for the characterisation of small molecule CRMs. The principles of exact matching (EM)-IDMS have been applied to absolute quantification of proteins based on proteolytic (most

Page 132 of 162

commonly tryptic) digestion of the protein, the use of isotopically labelled peptides as internal standards and of synthetic unlabelled peptides as primary standards. Isotopically labelled and unlabelled peptides are more readily available, and less expensive than isotopically labelled proteins, and are better characterised. Application to allergen proteins is difficult and costly, but once achieved can be cascaded via reference materials and certified reference materials so that the outcomes should be available at modest cost to support routine analysis.

It is recommended that an international programme be initiated leading to reference measurement methods for allergen proteins which provide results traceable to the SI. The methods should be applicable to the major allergens at clinically relevant concentrations (Table 8) in processed foods covering an appropriate range of protein, lipid and carbohydrate composition.

8.4.7 International collaboration

National support from food authorities, business organisations and National Measurement Institutes for the above recommendations is important, however international coordination is essential. The European Union legislates for the largest number of priority food allergen groups. Within the European Commission, the Health and Food Safety Directorate, DG Santé, is responsible for protection and improvement of public health, ensuring Europe's food is safe and wholesome and that citizens can be confident that their interests are protected. Work is already underway by several bodies, including individual National Measurement Institutes, (including LGC, the Joint Research Centre (JRC) of the European Commission and NIST), the MoniQA Association and iFAAM. Collating the various global work streams is needed to focus on the interrelated areas of analytical work that we have identified. This must be done in a transparent manner to achieve the aspirations of all stakeholders. DG Santé fulfils the criteria suggested by the above analysis. ***Thus the above recommendations were primarily addressed to DG Santé which should work closely with relevant bodies outside Europe to avoid duplication of effort or gaps.***

Food allergy is an increasing problem for all stakeholders and the food supply chain has been shown to be vulnerable to fraud involving food allergens, risking fatalities and reputational damage to the food industry (§ 5.2.2, 6 and 8.4.1 above). Legislation, risk assessment and risk management of food allergens show a high dependency on the ability to detect food allergens and quantitatively determine them. All current analytical approaches exhibit described deficiencies that jeopardise accurate results and risk false positives and false negatives. If we fail to realise the promise of many strands of risk assessment and risk management of food allergens through lack of the ability to measure food allergens reproducibly and with traceability, the analytical community will have failed a significant societal challenge. The recommendations made above parallel that which already pertains for the regulation by analysis of food additives.

It is clear that there are significant problems still to be solved, for example do we know if proteins purchased as a starting step in an analytical investigation really mimic the allergenic proteins e.g. as regards post translational modification, PTM, and tertiary structure? However, with work on these and all the strands outlined herein progress can be made. Calibrants are needed such as gravimetrically prepared peptide solutions with known concentrations traceable to the SI, or a solution of a well characterised protein of known concentration traceable by way of peptides to the SI. But how will these relate to a matrix reference material, say a food such as light roasted defatted peanut incurred in an industrially relevant matrix at a clinically relevant concentration? Figure 23 below illustrates in a highly simplified manner how this might be accomplished. For some food allergens clinical and bioinformatics studies have already identified relevant markers or allergenic proteins for which signature peptides are available. But this remains to be accomplished for all the major allergens. With the identification of the major relevant proteins of an allergenic food, and characterisation of the impact of food processing, analytical extraction, PTM, and tertiary structure (none of these are trivial tasks) a reference material can be created by either of two related approaches:

- a) A '*chimera*' theoretical matrix RM containing, in an industrially relevant matrix, clinically relevant concentrations of the optimal number of the separate component allergenic proteins of the food allergen already individually characterised and traceable to the SI by isotope dilution MS of the signature peptides, Cryar *et al.*, [13] following the recommendations of Johnson *et al.* 2011 or
- b) An empirical matrix RM containing, in an industrially relevant matrix, clinically relevant concentrations of mixed proteins extracted and characterised as described by van Eckert *et al.* 2006 and further traceable to the SI by investigations described in (a).

Figure 23 also indicates by way of a 'traffic light' code current progress towards the above goals; 'green' (i.e. accomplished), 'amber' (i.e. under way) or 'red' (i.e. yet to be done). The recommendations above are of a complexity and resource demand that only an internationally coordinated effort can accomplish them. However, rarely has such an exciting interdisciplinary scientific endeavour arisen as a solution to a key socially relevant problem.

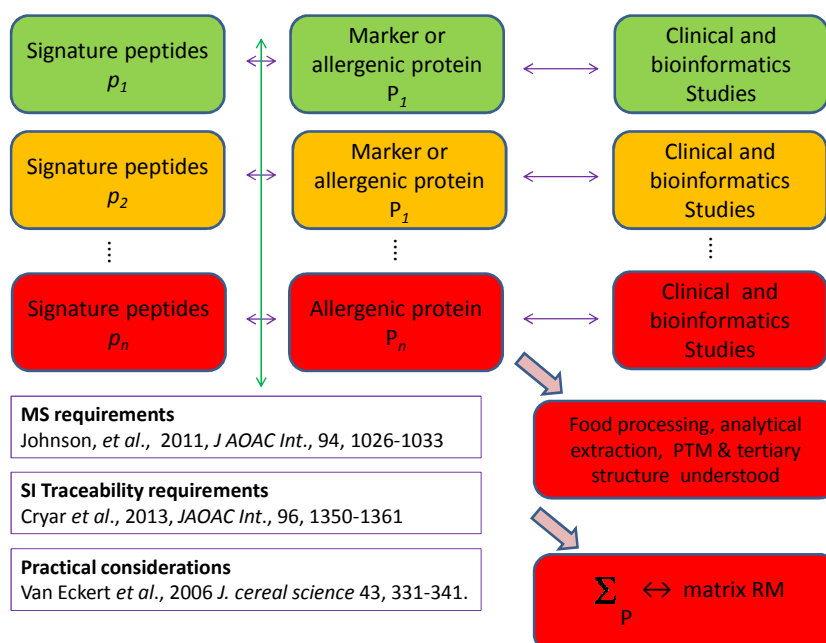


Figure 23: Simplified diagrammatic 'traffic light' illustration of work required to produce food allergen reference materials

9 References

- Accum F (1820) Treatise on adulterations of articles of food and drink. Longman, London
- Adulteration of Food and Drugs Act. 1872, 35 and 36 Victoria, c.74
- Allen, K.J., Remington, B.C., Baumert, J.L., Crevel, R.W., Houben, G.F., Brooke-Taylor, S., Kruizinga, A.G. and Taylor, S.L., 2014, Allergen reference doses for precautionary labeling (VITAL 2.0): clinical implications. *Journal of Allergy and Clinical Immunology* **133**(1), 156–164.
- An Act for Preventing the Adulteration of Articles of Food or Drink”, Victoria, c. 84, 1860
- Akyüz, M., 2008, Simultaneous determination of aliphatic and aromatic amines in ambient air and airbourne particulate matters by gas chromatography-mass spectrometry”, *Atmos. Environ.*, **42**, 3809-3819
- Akyüz M. and Ata, S., 2000, Simultaneous determination of aliphatic and aromatic amines in water and sediment samples by ion pair extraction and gas chromatography-mass spectrometry, *J. Chromat.*, **1129**, 88-94.
- Anagnostou, K., Islam, S., King, Y., Foley, L., Pasea, L., Bond, S., Palmer, C., Deighton, J., Ewan, P. and Clark, A., 2014, Assessing the efficacy of oral immunotherapy for the desensitisation of peanut allergy in children (STOP II): a phase 2 randomised controlled trial. *The Lancet* 383(9925), 1297–1304.
- Anagnostou, K. and Clark, A. (2015) The management of peanut allergy. *Archives of Disease in Childhood* **100**(1), 68–72.
- Anton R, Barlow S, Boskou D *et al.*, (2004) Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a Request from the Commission related to the use of certain food additives in Jelly mini cups. The EFSA Journal 82, 1–11 (and references therein).
- Arsene, CG., Ohlendorf, R., Burkitt, W., Pritchard C., Henrion A., O'Connor G., Bunk DM., and Guttler B., 2008, Protein Quantification by Isotope Dilution Mass Spectrometry of Proteolytic Fragments: Cleavage Rate and Accuracy, *Anal Chem*, **80**, 4154 - 4160
- .Arslan, M. M., Zeren C., Aydin Z., Akcan R., Dokuyucu R., Keten A. and Cekin N., 2015 Analysis of methanol and its derivatives in illegally produced alcoholic beverages. *J. Forensic and Legal Med.*, **33**, 56-60.
- Atwood, Thompson and Willett (2009): Atwood B., Thompson K. and Willett C., 2009, `Food Law: Third Edition, Tottel Publishing Ltd.
- Avery NJ., King RM., Knight S. and Hourihane J. O'B., 2003, Assessment of quality of life in children with peanut allergy, *Pediatr. Allergy Immunol.*, **14**, 378–382.
- Barnett, J., Muncer, K., Leftwich, J., Shepherd, R., Raats, M.M., Gowland, M.H., Grimshaw, K. and Lucas, J.S., 2011, Using 'may contain' labelling to inform food choice: a qualitative study of nut allergic consumers. *BMC Public Health*, **11**, 734.

- Barnett, J., Vasileiou, K., Gowland, MH., Raats, MM. and Lucas, JS., 2013, Beyond labelling: What strategies do nut allergic individuals employ to make food choices? A qualitative study. *PloS One*, **8**, e55293.
- Bauman, HE., 1995. The origin and concept of HACCP. In *HACCP in Meat, Poultry, and Fish Processing* (pp. 1-7), Springer, US.
- Baynes, RE., 2010 Human Health Risk Assessment, in Hodgson E., (Ed) A Textbook of Modern Toxicology, 4th Edition, Chapter 23, pp 489–505.
- Besler, M., 2001. Determination of allergens in foods. *TrAC Trends in Analytical Chemistry*, **20**, 662-672.
- Bindslev-Jensen, C., Ballmer-Weber, BK., Bengtsson, U., Blanco, C., Ebner, C., Hourihane, J., Knulst, AC., Moneret-Vautrin, DA., Nekam, K., Niggemann, B., Osterballe, M., Ortolani, C., Ring, J., Schnopp, C. and Werfel, T., 2004, Standardization of food challenges in patients with immediate reactions to foods—position paper from the European Academy of Allergology and Clinical Immunology. *Allergy* **59**, 690–697.
- Bosin TR. And Faull KF., 1988, Measurement of beta-carbolines by high performance liquid chromatography with fluorescence detection, *J. Chromat.*, **428**, 229-236.
- Borzelleca, JF., 2000, Paracelsus: herald of modern toxicology. *Toxicological Sciences* **53**(1), 2–4.
- Boye, JI., and Godefroy, SB., 2010, (Eds), Allergen Management in The Food Industry, Wiley, Hoboken, New Jersey.
- Boye, JI., 2012, Food allergies in developing and emerging economies: need for comprehensive data on prevalence rates, *Clinical and Translational Allergy*, **2**, 25.
- Burns, D. Thorburn, 1993, Precursors to and the Evolution of Elemental Organic Tube Combustion Analysis over the last Two Hundred Years”, *Analyt. Proc.*, **30**, 272-275.
- Charnley, B., 2005, Dr. Arthur Hill Hassall, the Analytical Sanitary Commission and the origins of food analysis: a re-examination of the 'food adulteration crisis' in the 1850's. Unpublished MA Thesis (University of Leeds), <http://berrischarnley.com/pdfs/charnley-ma-dissertation-2005.pdf> (accessed 30.07.2016)
- Charnley, B., 2008. Arguing over adulteration: the success of the Analytical Sanitary Commission. *Endeavour*, **32**, pp.129-133.
- BRC 2015: British Retail Consortium, BRC, 2015, BRC Global Standard for Food Safety Issue 7. Available at <http://www.brcglobalstandards.com/Home.aspx> (accessed 05.06.2016).
- Breiteneder, H. and Chapman, MD., 2014, Allergen nomenclature, In, Allergens and Allergen Immunotherapy: Subcutaneous, Sublingual, and Oral, 5th Ed., Eds Lockey, Richard F. and Ledford Dennis K. pp.37–49, CRC Press.
- Brottveit M and Lundin KE., 2008, Cancer risk in coeliac disease, Tidsskr. Nor. geforen: tidssk. prat. med., ny r kke, **128**(20), 2312 - 2315.
- Bucchini, L., Guzzon, A., Poms, R. and Senyuva, H., 2016, Analysis and critical comparison of food allergen recalls from the EU, USA, Canada, Hong Kong, Australia and New Zealand. *Food Additives and Contaminants, Part A*, in press.

- Burks, AW., Tang, M., Sicherer, S., Muraro, A., Eigenmann, PA., Ebisawa, M., Fiocchi, A., Chiang, W., Beyer, K., Wood, R. and Hourihane, J., 2012, ICON: Food allergy. *Journal of Allergy and Clinical Immunology* **129**, 906–920.
- Burnett, J., 1958, The History of Food Adulteration in Great Britain in the 19th Century, London PhD typescript, *not seen but cited by* Hobsbawm, E.J., 1963 (see below).
- Burns, D Thorburn, 2007, Alexander Wynter Blyth (1844-1921). A pioneering and innovative Public Analyst, *Journal of the Association of Public Analysts*, **35**, 17 - 29
- Caubet, JC., Ford, LS., Sickles, L., Järvinen, KM., Sicherer, SH., Sampson, HA. and Nowak-Węgrzyn, A., 2014, Clinical features and resolution of food protein-induced enterocolitis syndrome: 10-year experience. *Journal of Allergy and Clinical Immunology*, **134**, 382–389.
- Chafen, JJS., Newberry, SJ., Riedl, MA., Bravata, DM., Maglione, M., Suttorp, MJ., Sundaram, V., Paige, NM., Towfigh, A., Hulley, BJ. and Shekelle, PG., 2010, Diagnosing and managing common food allergies: a systematic review. *Journal of the American Medical Association*, **303**, 1848–1856.
- Chan, A.W.M., Chan J.K.C., Lee, T.H., Leung, T.F. and Tam, A.Y.C. (2015a) Guidelines for Allergy Prevention in Hong Kong. Available at <http://allergy.org.hk/> (accessed 26.03.2016)
- Chan, YT., Ho, HK., Lai, CK., Lau, CS., Lau, YL., Lee, TH., Leung, TF., Wong, GW. and Wu, YY., 2015b, Allergy in Hong Kong: an unmet need in service provision and training. *Hong Kong Medical Journal*, **21**, 52–60.
- Chen, J., Hu, Y., Allen, KJ., Ho, MH. and Li, H., 2011, The prevalence of food allergy in infants in Chongqing, China. *Pediatric Allergy and Immunology*, **22**, 356–360.
- Clare P and Clare M, 2012, The Life and Times of Alfred Henry Allen, Sheffield's First Public Analyst , *Journal of the Association of Public Analysts*, **40**, 39 – 59.
- Cochrane, SA., Salt, LJ., Wantling, E., Rogers, A., Coutts, J., Ballmer-Weber, BK., Fritsche, P., Fernández-Rivas, M., Reig, I., Knulst, A. and Le, TM., 2012. Development of a standardized low-dose double-blind placebo-controlled challenge vehicle for the EuroPrevall project. *Allergy*, **67**, 107-113.
- Codex Alimentarius 2010: *Codex Alimentarius* Commission, General Standard for the Labelling of Prepackaged Foods. CODEX STAN 1-1985 (1985, last amended 2010).
- Collin P, Thorell L, Kaukinen K and Mäki M,. 2004, The safe threshold for gluten contamination in gluten-free products. Can trace amounts be accepted in the treatment of celiac disease? *Alimentary Pharmacology & Therapeutics*, **19**, 1277-1283.
- Cree L. and Reid, M., 2009, Development of the UK food samples surveillance system, *Public Health*, **123**, 89-94.
- Crevel, RW., Baumert, JL., Baka, A., Houben, GF., Knulst, AC., Kruizinga, AG., Luccioli, S., Taylor, SL. and Madsen, CB., 2014a, Development and evolution of risk assessment for food allergens. *Food and Chemical Toxicology*, **67**, 262–276.
- Crevel, RW., Baumert, JL., Luccioli, S., Baka, A., Hattersley, S., Hourihane, J.O.B., Ronsmans, S., Timmermans, F., Ward, R. and Chung, YJ., 2014b, Translating

reference doses into allergen management practice: Challenges for stakeholders. *Food and Chemical Toxicology* **67**, 277–287.

Crevel, RW., 2015, Food allergen risk assessment and management, *in* Handbook of Food Allergen Detection and Control, S. Flanagan (Ed), Chapter 3, pp. 41–66

De Leon, MP., Rolland, JM. and O'Hehir, RE., 2007, The peanut allergy epidemic: allergen molecular characterisation and prospects for specific therapy. *Expert Reviews in Molecular Medicine*, **9**(01), 1–18.

Dhami, S., Panesar, SS., Roberts, G., Muraro, A., Worm, M., Bilò, MB., Cardona, V., Dubois, AEJ., DunnGalvin, A., Eigenmann, P. and Fernandez-Rivas, M., 2014, Management of anaphylaxis: a systematic review. *Allergy*, **69**, 168–175.

Diamond HA., 1990, Reasonable Doubt: To Define, or Not to Define, *Columbia Law Review*, **90**, 1716–1736.

DunnGalvin, A., Chan, C.H., Crevel, R., Grimshaw, K., Poms, R., Schnadt, S., Taylor, S.L., Turner, P., Allen, KJ., Austin, M. and Baka, A., 2015, Precautionary allergen labelling: perspectives from key stakeholder groups. *Allergy* **70**, 1039–1051.

Du Toit, G., Tsakok, T., Lack, S. and Lack, G., 2016a, Prevention of food allergy, *Journal of Allergy and Clinical Immunology* **137**, 998–1010.

Du Toit, G., Sayre, PH., Roberts, G., Sever, ML., Lawson, K., Bahnson, HT., Brough, HA., Santos, AF., Harris, K.M., Radulovic, S. and Basting, M. (2016b) Effect of avoidance on peanut allergy after early peanut consumption. *New England Journal of Medicine* **374**, 1435–1443.

Dunlap, FL, 1911, The food laws of the United Kingdom and their Administration US Department of Agriculture, Government Printing Office, Washington

Dyer B, Mitchell CA., 1932, The society of public analysts and other analytical chemists: some reminiscences of its first fifty years and a review of its activities. W. Heffer & Sons, Cambridge , Relations between public analysts and Somerset House chemists, pp 14–18

Van Eckert, R., Berghofer, E., Ciclitira, P.J., Chirido, F., Denery-Papini, S., Ellis, H.J., Ferranti, P., Goodwin, P., Immer, U., Mamone, G. and Mendez, E., 2006. Towards a new gliadin reference material—isolation and characterisation. *Journal of cereal science*, **43**, 331–341.

EFSA (European Food Safety Authority), 2005, Scientific Panel on Food Additives, Review of the toxicology of a number of dyes illegally present in food in the EU, *EFSA Journal*, **263**: 1–71.

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2013. Scientific Opinion on the re-evaluation of aspartame (E 951) as a food additive. *EFSA Journal* 2013;11(12):3496, 263 pp. doi:10.2903/j.efsa.2013.3496

EFSA, European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies (2014a) Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes. *EFSA Journal* **12**(11), 3894.

- EFSA, European Food Safety Authority (2014b) Modern methodologies and tools for human hazard assessment of chemicals. *EFSA Journal* **12**(4), 3638.
- European Parliament and Council 1994 Directive 94/35/EC of 30 June 1994 on sweeteners for use in foodstuffs, OJ L 237, 10.9.1994, p. 3 see <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:%2031994L0035:EN:NOT> for the original text, consolidated versions and amendments (accessed 07 05 10)
- European Parliament and Council, 1995, Directive No 95/2/EC of 20 February 1995 on food additives other than colours and intense sweeteners OJ L 61, 18.3.1995, p. 1–40 see <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31995L0002:en:NOT> for the original text, consolidated versions and amendments (accessed 07 05 10)
- European Union 2004: Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, OJ L 165, 30.4.2004, p. 1, see Eur Lex for latest consolidated version.
- European Union 2008a: Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings, OJ 31.12.2008, L354/1
- European Union 2008b: Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, OJ L 354/16 - L 354/33
- Feng F., Zhao Y., Yong W., Sun L., Jiang G. and Chu X., 2011, Highly sensitive and accurate screening of 40 dyes in soft drinks by liquid chromatography-electrospray tandem mass spectrometry, *J Chromat* **879** B: 1813 - 1818
- Fiedler, W., Borchers, C., Macht, M., Deininger, SO. and Przybylski, M., 1998, Molecular characterization of a conformational epitope of hen egg white lysozyme by differential chemical modification of immune complexes and mass spectrometric peptide mapping. *Bioconjugate chemistry*, **9**(2), pp.236 - 241.
- Flanagan, S. 2015 Assessment and communication of allergen risks in the food chain, *In* Flanagan, S. (Ed.) *Handbook of Food Allergen Detection and Control*, Woodhead Publishing 2015, p.67.
- Fournier M., Lesage J., Ostiguy C. and Tra HV, 2008, Sampling and analytical methodology development for the determination of primary and secondary low molecular weight amines in ambient air, *J. Environ. Monit.*, **10**, 379 - 386
- French, M. and Phillips, J., 2000. *Cheated not poisoned?: food regulation in the United Kingdom, 1875-1938*. Manchester University Press
- The Food Safety (Sampling and Qualifications) (England) Regulations 2013 SI 264
 The Food Safety (Sampling and Qualifications) (Scotland) Regulations 2013 SSI 84
 The Food Safety (Sampling and Qualifications) (Wales) Regulations 2013 WSI 479 (W.55)
 The Food Safety (Sampling and Qualifications) Regulations (Northern Ireland) 2013 NISR 66
- Food Standards Agency, FSA, 2002, Food Additives Legislation Guidance Notes. Available at <http://www.food.gov.uk/multimedia/pdfs/guidance.pdf> (Accessed 07 05 10)

- Food Standards Agency, FSA (2003) Food Standards Agency. Available at <http://tna.europarchive.org/20110116113217/http://www.food.gov.uk/news/pressreleases/2003/jul/bannedsweets> (last accessed 01.08.2016)
- Food Standards Agency. (2004) Jelly mini-cups. Available at <http://tna.europarchive.org/20110116113217/http://www.food.gov.uk/news/newsarchive/2004/apr/jellysweetseubannews> (Accessed 01.08.2016)
- Food Standards Agency, FSA, UK Food Surveillance System (UKFSS) <http://www.food.gov.uk/enforcement/monitoring/fss/> (accessed 09 05 10)
- Food Standards Agency, FSA, (2006) Guidance on Allergen Management and Consumer Information. Available at <http://www.food.gov.uk/sites/default/files/multimedia/pdfs/maycontainguide.pdf> (Accessed 02.07.2016)
- Food Standards Agency, FSA, 2002, Food Additives Legislation Guidance Notes. Available at <http://www.food.gov.uk/multimedia/pdfs/guidance.pdf> (Accessed 07.05.2010)
- Food Standards Agency, FSA, Report of the Sudan 1 Review Panel, July 2007, available at <http://tna.europarchive.org/20120419000433/http://www.food.gov.uk/multimedia/pdfs/board/fsa070906.pdf> (accessed 01.08.2016)
- Food Standards Agency, UK, 2009, 'Aspartame study to begin' <http://www.food.gov.uk/news/newsarchive/2009/jun/aspartame> (accessed 18 April 2010)
- Food Standards Agency, UK, FSA 2009a: Quarterly Public Attitudes Tracker: December 2009 Food Standards Agency 22 December 2009 available at: <http://www.food.gov.uk/multimedia/pdfs/trackerdecem09.pdf> (accessed 03 04 2010)
- Food Standards Agency, UK, FSA 2009b: See list of annual consumer attitude reports available at <http://www.food.gov.uk/science/socsci/surveys/foodsafety-nutrition-diet/> (accessed 03.04.10)
- Food Standards Agency, UK, FSA 2009c: Food Standards Agency, UK, 2009, 'Aspartame study to begin' <http://www.food.gov.uk/news/newsarchive/2009/jun/aspartame> (accessed 18 April 2010)
- Food Standards Agency, UK FSA (2010) 'Southampton' colours labelling <https://www.food.gov.uk/business-industry/guidancenotes/labelregsguidance/labellingfoodcoloursreg13332008> (accessed 02.08.2016)
- Food Standards Agency, UK FSA 2016, Food Standards Agency, Biannual Public Attitudes Tracker, Wave 11, November 2015, Social Science Research Unit January 2016, available at: <https://www.food.gov.uk/sites/default/files/tracker-wave-11.pdf> (accessed 01.08.2016)
- Food Standards Agency, FSA, (2016a) Allergy and intolerance. Available at <http://www.food.gov.uk/science/allergy-intolerance> (Accessed 02.07.2016)
- García, E., Llorente, M., Hernando, A., Kieffer, R., Wieser, H and Méndez, E., 2005, Development for a general procedure for the complete extraction of gliadins for heat

- processed and unheated foods, *European Journal of Gastroenterology & Hepatology*, **17**, 529 - 539.
- Gélinas P., McKinnon CM., Mena MC. and Méndez E., 2008, Gluten contamination of cereal foods in Canada, *International Journal of Food Science & Technology*, **43**, 1245-1252
- Gendel, SM., 2012, Comparison of international food allergen labelling regulations. *Regulatory Toxicology and Pharmacology* **63**, 279–285.
- Gibbison B., Sheikh A., McShane P., Haddow C. and Soar, J., 2012, Anaphylaxis admissions to UK critical care units between 2005 and 2009, *Anaesthesia*, **67**, 833-839
- Gowland, MH., Ledford, CL. and Austin, MM., 2012, Which food allergies are most common in the UK?-using anaphylaxis campaign member data and case files to indicate prevalence. *Clinical and Experimental Allergy* **42**, 1834 – 1835.
- Gowland, MH. and Walker, M.J., 2014, Food Allergy, a summary of 8 cases in the UK criminal and civil courts: effective last resort for vulnerable consumers? *Journal of the Science of Food and Agriculture* **95**, 1979 – 1990.
- Gray, KM., Walker, MJ., Burn, MJS., Mazur, M., Niedzwiedzka, K., Liszka, K. and Burns, DT., 2016, Illegal Dyes in Food and Spices—A 2006 LGC LC-UV/Visible Method Reviewed and Updated for 19 Dyes. *Journal of the Association of Public Analysts (Online)*, **44**, 018 - 039.
- Green PHR and Jabri B., 2003, Coeliac disease, *The Lancet*, **362**(9381), 383 - 391.
- GSFA 2011: Codex General Standard for Food Additives, Online Database, Codex STAN 192-1995, INS number: 433, FAO
- Hamano T., Mitsuhashi Y. and Matsuki Y., 1981, Glass capillary gas chromatography of secondary amines after derivatisation with benzenesulfonyl chloride", *Agric. Biol. Chem.*, **45**, 2237-2243.
- Hammond, PW. and Egan, H., 1992, Weighed in the Balance, A history of the Laboratory of the Government Chemist, HMSO, Chapter 5, pp 86 – 100.
- Hassall AH, 1855, Food and its adulterations, comprising reports of the Analytical Sanitary Commission of the Lancet for the years 1851–1854, revised and extended, London
- Hattersley, S., Ward, R., Baka, A. and Crevel, RW., 2014, Advances in the risk management of unintended presence of allergenic foods in manufactured food products—an overview. *Food and Chemical Toxicology* **67**, 255–261.
- Health Canada, 2012, The use of food allergen precautionary statements on prepackaged foods. Available at http://www.hc-sc.gc.ca/fn-an/label-etiquet/allergen/precaution_label-etiquette-eng.php (Accessed 02.07.2016).
- Hirst, B. (2014) Survey of allergen labelling and allergen content of processed foods, and final report survey of allergen advisory labelling and allergen content of UK retail pre-packed processed foods, FS241038 (T07067), 4 November 2014, retrieved from Food Standards Agency. Available at <https://www.food.gov.uk/sites/default/files/survey-allergen-labelling-prepacked.pdf> (Accessed 02.07.2016).

- HMSO, the Food Labelling Regulations 1996, Statutory Instrument 1996 No. 1499, Regulation 2.
- HMSO, the Food Safety (Sampling and Qualifications) Regulations 1990, Statutory Instrument 1990 No. 2463
- Hobsbawm, E.J., 1963. The standard of living during the Industrial Revolution: a discussion. *The Economic History Review*, **16**, 119 - 146.
- Hochegger, R., Mayer, W. and Prochaska, M., 2015. Comparison of R5 and G12 Antibody-Based ELISA Used for the Determination of the Gluten Content in Official Food Samples. *Foods*, **4**, 654 - 664.
- Hodgson, E, (Ed) A Textbook of Modern Toxicology, 4th Edition, 2010, John Wiley and Sons, Inc., Hoboken, New Jersey.
- Hood, C., Rothstein, H., Baldwin, R., Rees, J. and Spackman, M., 1999. Where risk society meets the regulatory state: Exploring variations in risk regulation regimes. *Risk Management*, **1**, 21 – 34.
- Hotchkiss JH. and Vecchio AJ., 1983, Analysis of direct contact paper and paperboard food packaging for N-nitrosomorpholine and morpholine", *J. Food Sci.*, **48**, 240 – 241.
- Houben, G., Burney, P., Chan, CH., Crevel, R., Dubois, A., Faludi, R., Entink, RK., Knulst, A., Taylor, S. and Ronsmans, S., 2016, Prioritisation of allergenic foods with respect to public health relevance: Report from an ILSI Europe Food Allergy Task Force Expert Group. *Food and Chemical Toxicology* **89**, 8 – 18.
- Hourihane, JO'B., Bedwani, SJ., Dean, TP. and Warner, JO., 1997a, Randomised, double blind, crossover challenge study of allergenicity of peanut oils in subjects allergic to peanuts. *British Medical Journal* **314**(7087), 1084 – 1088.
- Hourihane, J.O'B., Kilburn, SA., Nordlee, JA., Hefle, SL., Taylor, SL. and Warner, JO., 1997b, An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut protein: a randomized, double-blind, placebo-controlled food challenge study. *Journal of Allergy and Clinical Immunology* **100**, 596 – 600.
- Hourihane, JO'B., 2001, The threshold concept in food safety and its applicability to food allergy. *Allergy* **56**(s67), 86–90.
- HSE 2007: Health and Safety Executive, Workplace exposure limits, EH40/2007.
- IPCS 1995: Morpholine, Health and Safety Guide, IPCS International Programme on Chemical Safety, Health and Safety Guide No. 92, United Nations Environment Programme, International Labour Organisation, Geneva,(1995).
- Igea, JM., 2013, The history of the idea of allergy. *Allergy* **68**, 966 – 973.
- Järvinen, .M. and Nowak-Węgrzyn, A., 2013, Food protein-induced enterocolitis syndrome (FPIES): current management strategies and review of the literature. *The Journal of Allergy and Clinical Immunology: In Practice* **1**, pp.317 – 22.
- JECFA 1973: Joint FAO/WHO Expert Committee on Food Additives, Specification for polyoxyethylene (20) sorbitan monooleate, Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992).

- JECFA 1989: Joint FAO/WHO Expert Committee on Food Additives, Turmeric Oleoresin, Prepared at the 35th JECFA (1989), published in FNP 49 (1990) and in FNP 52 (1992). Retrieved from the Food and Agriculture Organisation of the United Nations, FAO, Online Edition: "Combined Compendium of Food Additive Specifications"
- Johnson, P.E., Baumgartner, S., Aldick, T., Bessant, C., Giosafatto, V., Heick, J., Mamone, G., O'connor, G., Poms, R., Popping, B. and Renter, A., 2011. Current perspectives and recommendations for the development of mass spectrometry methods for the determination of allergens in foods. *Journal of AOAC International*, **94**, 1026 – 1033.
- Johansson, SGO., Hourihane, JO'B., Bousquet, J., Bruijnzeel-Koomen, C., Dreborg, S., Haahtela, T., Kowalski, ML., Mygind, N., Ring, J., Van Cauwenberge, P. and Hage-Hamsten, V., 2001, A revised nomenclature for allergy: an EAACI position statement from the EAACI nomenclature task force. *Allergy* **56**, 813 – 824.
- Johansson, SGO., 2016, The discovery of IgE. *Journal of Allergy and Clinical Immunology* **137**, 1671 – 1673.
- Kataoka H., Shindoh S. and Makita M., 1995, Determination of secondary amines in various foods by gas chromatography with flame ionisation detection, *J. Chromat. A*, **695**, 142-148.
- Kawawa, N., 2013, Jelly Mini-Cups Containing Konjac: Is a Warning Enough to Protect Vulnerable Consumers?. *Australian Journal of Asian Law* 13.2
- Kennedy, NP. and Feighery, C., 2000, Clinical features of coeliac disease today. *Biomedicine and Pharmacotherapy* **54**, 373 – 380.
- Kim, SS., 2014, The Mini-Cup Jelly Court Cases: A Comparative Analysis from a Food Ethics Perspective." *Journal of Agricultural and Environmental Ethics* **27**, 735 - 748.
- . King RM., Knibb RC. and. Hourihane J.O'B, 2009, Impact of peanut allergy on quality of life, stress and anxiety in the family. *Allergy*, **64**, 461–468.
- Kjeldahl J., 1883, "Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern", *Z. Analyt. Chem.* **22**, 366-382.
- Kruizinga, A.G., Briggs, D., Crevel, R.W.R., Knulst, A.C., Van den Bosch, L.M.C. and Houben, G.F., 2008. Probabilistic risk assessment model for allergens in food: sensitivity analysis of the minimum eliciting dose and food consumption. *Food and Chemical Toxicology*, **46**(5), pp.1437-1443.
- Law, M.T., 2003. The origins of state pure food regulation. *The Journal of Economic History*, **63**, 1103-1130.
- Law Commission 2009: The Admissibility of Expert Evidence in Criminal Proceedings in England and Wales, A New Approach to the Determination of Evidentiary Reliability, The Law Commission, Consultation Paper No 190, 2009
- Lee, A.J., Thalayasingam, M. and Lee, B.W. (2013) Food allergy in Asia: how does it compare? *Asia Pacific Allergy* **3**(1), 3–14.
- Lehotay, S.J., Mastovska, K., Amirav, A., Fialkov, A.B., Martos, P.A., de Kok, A. and Fernández-Alba, A.R., 2008. Identification and confirmation of chemical residues in

food by chromatography-mass spectrometry and other techniques. *TrAC Trends in Analytical Chemistry*, 27(11), pp.1070-1090.

- Leitch, I., Blair, I.S. and McDowell, D.A., 2001. The role of environmental health officers in the protection of allergic consumers. *International journal of environmental health research*, 11(1), pp.51-61.
- Lester D R (2008) Gluten measurement and its relationship to food toxicity for celiac disease patients, *Plant Methods*, 4: 26.
- Leung, T.F., Yung, E., Wong, Y.S., Lam, C.W. and Wong, G.W. (2009) Parent-reported adverse food reactions in Hong Kong Chinese pre-schoolers: epidemiology, clinical spectrum and risk factors. *Pediatric Allergy and Immunology* 20(4) 339–346.
- Lindahl R., Wästerby A. and Levin J. O., (2001). Determination of morpholine in air by derivitisation with 1-naphthylisocyanate and HPLC analysis, *Analyst*, 126, 152-154
- Lomer, M.C.E. (2015) Review article: the aetiology, diagnosis, mechanisms and clinical evidence for food intolerance. *Alimentary Pharmacology and Therapeutics* 41, 262–275.
- MacDonald Ian and Hulme Amanda, 2000, *Food Standards Regulation, The New Law*. Jordans, Bristol.
- Maizels M. and Budde W. L, 2001, Exact Mass Measurements for Confirmation of Pesticides and Herbicides Determined by Liquid Chromatography/Time-of-Flight Mass Spectrometry", *Analyt. Chem.*, 73, 5436-5440.
- Marchisotto, M.J., Harada, L., Blumenstock, J., Bilaver, L., Wasserman, S., Sicherer, S., Boloh, Y., Regent, L., Said, M., Schnadt, S. and Allen, K. (2016) Global Perceptions of Food Allergy Thresholds in 16 Countries. *Allergy* DOI: 10.1111/all.12933.
- McCann D, Barrett A, Cooper A, Crumpler D, Dalen L, Grimshaw K, Kitchen E, Lok K, Porteous L, Prince E, Sonuga-Barke E, Warner JO & Stevenson J (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo controlled trial. *Lancet*, 370, 1560-1567.
- McCann D, Barrett A, Cooper A, Crumpler D, Dalen L, Grimshaw K, Kitchen E, Lok K, Porteous L, Prince E, Sonuga-Barke E, Warner JO & Stevenson J (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo controlled trial. *Lancet*, 370, 1560-1567.
- McGough N (2009) Coeliac Disease: allergy or intolerance? in Coutts J and Fielder R (eds) *Management of Food Allergens*, Wiley-Blackwell, Oxford, chap. 4.
- McIntosh, J., Flanagan, A., Madden, N., Mulcahy, M., Dargan, L., Walker, M. and Burns, D.T. (2011) Awareness of coeliac disease and the gluten status of 'gluten-free' food obtained on request in catering outlets in Ireland. *International Journal of Food Science and Technology* 46, 1569–1574.
- B. McClean, 2007. Meat and meat products: the calculation of meat content, added water and connective tissue content from analytical data, Campden & Chorleywood Food Research Association, Chipping Campden.

- Madsen, C. B., Hattersley, S., Allen, K. J., Beyer, K., Chan, C.-H., Godefroy, S. B., Hodgson, R., Mills, E.N., Muñoz-Furlong, A, Schnadt, S., Ward, R., Wickman, M. and Crevel, R. (2012) Can we define a tolerable level of risk in food allergy? Report from a EuroPrevall/UK Food Standards Agency workshop. *Clinical and Experimental Allergy* **42**, 30–37.
- Mahesh, P.A., Wong, G.W., Ogorodova, L., Potts, J., Leung, T.F., Fedorova, O., Holla, A.D., Fernandez-Rivas, M., Clare Mills, E.N., Kummeling, I. and Versteeg, S.A. (2016) Prevalence of food sensitization and probable food allergy among adults in India: the EuroPrevall INCO study. *Allergy* **71**, 1010–1019.
- Medline Plus, U.S. National Library of Medicine, National Institutes of Health, 2010, Phenylketonuria, Available at: <http://www.nlm.nih.gov/medlineplus/ency/article/001166.htm> (accessed 07 05 10)
- Millard, P., Paine, S., O'Hagan, S. and Hipkiss, J. (2015) Traceability of allergenic foods in the food chain. In Flanagan, S. (Ed.) *Handbook of Food Allergen Detection and Control*, Woodhead Publishing, pp.19.
- Moissidis, I., Chaidaroon, D., Vichyanond, P. and Bahna, S. L. (2005) Milk-induced pulmonary disease in infants (Heiner syndrome). *Pediatric Allergy and Immunology* **16**, 545–552
- Muraro A. , Werfel T., Hoffmann-Sommergruber K., Roberts G., Beyer K., Bindslev-Jensen C., Cardona V., Dubois A., du Toit G., Eigenmann P., *et al.*, on behalf of the EAACI Food Allergy and Anaphylaxis Guidelines Group, EAACI Food Allergy and Anaphylaxis Guidelines. Diagnosis and management of food allergy. *Allergy* 2014, **69**, 1008–1025.
- Muraro, A., Roberts, G., Worm, M., Bilò, M.B., Brockow, K., Fernández Rivas, M., Santos, A.F., Zolkipli, Z.Q., Bellou, A., Beyer, K., Bindslev-Jensen, C., Cardona, V., Clark, A.T., Demoly, P., Dubois, A.E.J., DunnGalvin, A., Eigenmann, P., Halken, S., Harada, L., Lack, G., Jutel, M., Niggemann, B., Ruëff, F., Timmermans, F., Vlieg-Boerstra, B.J., Werfel, T., Dhami, S., Panesar, S., Akdis, C.A., Sheikh, A. (2014a) Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. *Allergy* **69**, 1026–1045.
- Muraro, A., Hoffmann-Sommergruber, K., Holzhauser, T., Poulsen, L.K., Gowland, M.H., Akdis, C.A., Mills, E.N.C., Papadopoulos, N., Roberts, G., Schnadt, S. and Ree, R. (2014b) EAACI Food Allergy and Anaphylaxis Guidelines. Protecting consumers with food allergies: understanding food consumption, meeting regulations and identifying unmet needs. *Allergy* **69**, 1464–1472.
- Muraro, A., Halken, S., Arshad, S.H., Beyer, K., Dubois, A.E.J., Du Toit, G., Eigenmann, P.A., Grimshaw, K.E.C., Hoest, A., Lack, G., O'Mahony, L., Papadopoulos, N.G., Panesar, S., Prescott, S., Roberts, G., de Silva, D., Venter, C., Verhasselt, V., Akdis, A.C., Sheikh, A. (2014c) EAACI Food Allergy and Anaphylaxis Guidelines. Primary prevention of food Allergy. *Allergy* **69**, 590–601.
- Nikoleski , D, 2015, Hygienic design and cleaning as an allergen control measure, In Flanagan, S. (Ed.) *Handbook of Food Allergen Detection and Control*, Woodhead Publishing 2015, p.89.
- NIST 2008: The National Institute of Standards and Technology (NIST), Standard Reference Database 69 (2008).

NLM 2011: US National Library of Medicine Hazardous Substances Data Bank.

Nowak-Węgrzyn, A., Katz, Y., Mehr, S.S. and Koletzko, S. (2015) Non-IgE-mediated gastrointestinal food allergy. *Journal of Allergy and Clinical Immunology* **135**(5), 1114–1124.

NHS Choices: The truth about aspartame, <http://www.nhs.uk/Livewell/Goodfood/Pages/the-truth-about-aspartame.aspx> Page last reviewed: 13/04/2016 (Accessed 01.08.2016)

Nwaru, B.I., Panesar, S.S., Hickstein, L., Rader, T., Werfel, T., Muraro, A., Hoffmann-Sommergruber, K., Roberts, G. and Sheikh, A. (2013) The epidemiology of food allergy in Europe: protocol for a systematic review. *Clinical and Translational Allergy* **3**, 13.

Nwaru, B.I., Hickstein, L., Panesar, S.S., Muraro, A., Werfel, T., Cardona, V., Dubois, A.E.J., Halken, S., Hoffmann-Sommergruber, K., Poulsen, L.K., Roberts, G., Van Ree, R., Vlieg-Boerstra, B.J., Sheikh, A. (2014a) The epidemiology of food allergy in Europe: a systematic review and meta-analysis. *Allergy* **69**, 62–75.

Nwaru, B.I., Hickstein, L., Panesar, S.S., Roberts, G., Muraro, A. and Sheikh, A. (2014b) Prevalence of common food allergies in Europe: a systematic review and meta-analysis. *Allergy* **69**, 992–1007.

Oddy D.J., 2007, in 'Food and the City in Europe since 1800' Eds: Peter J Atkins, Peter Lummel, and Derek J Oddy, 2007, Ashgate Publishing Ltd, Hampshire, Chapter 8 Food Quality in London and the Rise of the Public Analyst, 1870 – 1939, pp 91 – 103

Oplatowska M, Stevenson P J, Schulz C, Hartig L, and Elliott CT, (2011), Development of a simple gel permeation clean-up procedure coupled to a rapid disequilibrium enzyme-linked immunosorbent assay (ELISA) for the detection of Sudan I dye in spices and sauces, *Anal Bioanal Chem*, 401: 1411 – 1422

Owen, L. and Gilbert, J., 2009. Proficiency testing for quality assurance of allergens methods, *Analytical and bioanalytical chemistry*, **395**,147-153.

Paik M-J., Choi Y. and Kim K-R., 2006, Simultaneous profiling analysis of alkylphenols and amines by gas chromatography and gas chromatography-mass spectrometry, *Analyt, Chim. Acta*, 560, 218-226,

Panesar, S.S., Javad, S., Silva, D.D., Nwaru, B.I., Hickstein, L., Muraro, A., Roberts, G., Worm, M., Bilo, M.B., Cardona, V. and Dubois, A.E.J. (2013) The epidemiology of anaphylaxis in Europe: a systematic review. *Allergy* **68**, 1353–1361.

Pearson 1991: R. S. Kirk and R. Sawyer (eds.), "Pearson's Composition and Analysis of Foods", 9th edition, Longman Scientific and Technical, Harlow. 1991

Perkin, M.R., Logan, K., Tseng, A., Raji, B., Ayis, S., Peacock, J., Brough, H., Marrs, T., Radulovic, S., Craven, J. and Flohr, C. (2016) Randomized trial of introduction of allergenic foods in breast-fed infants. *New England Journal of Medicine* **374**(18), 1733–1743.

B. Pfundstein, A. R. Tricker and R. Pressmann, (1991), Determination of primary and secondary amines in foods using gas chromatography and chemiluminescence detection with a modified thermal energy analyser, *J. Chromat.*, 539, 141-148

Points, J. Personal communication, January 2016

- Pardo O, Yusà V, León N, Pastor A, 2009, Development of a method for the analysis of seven banned azo-dyes in chilli and hot chilli food samples by pressurised liquid extraction and liquid chromatography with electrospray ionization-tandem mass spectrometry, *Talanta*, **78**, 178–186
- Prescott, S., and Allen, K.J., 2011, Food allergy: riding the second wave of the allergy epidemic. *Pediatric Allergy and Immunology* **22**, 155–160.
- Prescott, S.L., Pawankar, R., Allen, K.J., Campbell, D.E., Sinn, J.K., Fiocchi, A., Ebisawa, M., Sampson, H.A., Beyer, K. and Lee, B.W., 2013, A global survey of changing patterns of food allergy burden in children. *World Allergy Organization Journal*, **6**(1), 21.
- Pritchard C., Quaglia M., Mussell C., Burkitt W.I., Parks H., O'Connor G., 2009, Fully Traceable Absolute Protein Quantification of Somatropin that Allows Independent Comparison of Somatropin Standards, *Clin Chem*, , **55**, 1984-1990
- Quaglia M., Pritchard C., Hall Z. and O'Connor G., 2008, Amine-reactive Isobaric Tagging Reagents: Requirements for Absolute Quantification of Proteins and Peptides, , *Anal Biochem*, **379**, 26-31
- Quaglia M., O'Connor G., 2010, Getting the Best of Both Worlds: Ensuring the Perfect Partnership of Chromatographic Separation and MS Detection for Protein Quantification, , *Chromatography Today*, **3**(2), 11
- Quarterly Public Attitudes Tracker: December 2009 Food Standards Agency 22 December 2009 available at: <http://www.food.gov.uk/multimedia/pdfs/trackerdecem09.pdf> (accessed 03 04 2010)
- Qureshi S, Mink R., 2003. Aspiration of fruit gel snacks. *Pediatrics* **111**:687–689
- Raina VK, Srivastava SK, Jain N, Ahmad A, Syamasundar KV and Aggarwal KK, (2002) Essential oil composition of *Curcuma longa* L. cv. Roma from the plains of northern India, *Flav Frag J*, **17**: 99-102
- Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, OJ L 354/16 - L 354/33
- Rimbaud, L., Heraud, F., La Vieille, S., Leblanc, J.C. and Crepet, A., 2010, Quantitative risk assessment relating to adventitious presence of allergens in food: a probabilistic model applied to peanut in chocolate. *Risk Analysis*, **30**(1), 7–19.
- Rimbaud, L., Héraud, F., La Vieille, S., Leblanc, J.C. and Crépet, A., 2013, Quantitative risk assessment relating to the inadvertent presence of peanut allergens in various food products. *International Food Risk Analysis Journal* **3**, 1–11
- Rosell, C.M., Barro, F., Sousa, C. and Mena, M.C., 2014. Cereals for developing gluten-free products and analytical tools for gluten detection. *Journal of Cereal Science*, **59**, 354-364.
- Rothstein, H.F., 2004. Precautionary bans or sacrificial lambs? Participative risk regulation and the reform of the UK food safety regime. *Public Administration*, **82**(4), 857-881.
- Rothstein, H., 2005. Escaping the Regulatory Net: Why Regulatory Reform Can Fail Consumers. *Law & Policy*, **27**(4), 520-548.

- Rothstein, H., 2007. Talking shops or talking turkey? institutionalizing consumer representation in risk regulation. *Science, Technology & Human Values*, **32**(5), 582-607.
- Rothstein, H., 2013. Domesticating participation: participation and the institutional rationalities of science-based policy-making in the UK food standards agency. *Journal of Risk Research*, **16**(6), 771-790.
- RSC 2016: Royal Society of Chemistry, Information, including syllabus and examinations for the Mastership in Chemical Analysis <http://www.rsc.org/careers/cpd/practising-scientists/mastership-chemical-analysis/#mchema> (Accessed 28.07.2016)
- Safefood, a body responsible for the promotion of food safety in both jurisdictions of the island of Ireland, <http://www.safefood.eu/>
- Schroeder, H.W. and Cavacini, L. (2010) Structure and function of immunoglobulins. *Journal of Allergy and Clinical Immunology* **125**(2), S41–S52.
- Seidel JS, Gausche-Hill M (2002) Lychee-flavored gel candies, a potentially lethal snack for infants and children. *Arch Pediatr Adolesc Med* **156**:1120–1122
- Sen N. P. and Baddoo P. A., 1989, An investigation on the possible presence of morpholine and N-nitrosomorpholine in wax-coated apples”, *J. Food Safety*, **9**, 183-191.
- Sicherer, S.H. and Sampson, H.A. (2010) Food allergy. *Journal of Allergy and Clinical Immunology* **125**(2), S116–S125.
- Sicherer, S.H. and Sampson, H.A. (2014) Food allergy: epidemiology, pathogenesis, diagnosis, and treatment. *Journal of Allergy and Clinical Immunology* **133**(2), 291–307.
- Singer G. M. and Lijinsky W., 1976, Naturally occurring nitrosatable compounds. I, Secondary amines in foodstuffs, *J. Agric. Food Chem.*, **24**. 550-553
- da Silva Neves M M P, González-García M B, Nouws H P A, Delerue-Matos C, Santos-Silva A, and Costa-García A (2010) Celiac disease diagnosis and gluten free food analytical control, *Analytical & Bioanalytical Chemistry*, **397**(5), 1743-1753.
- Skerritt J H and Hill A S (1991) Enzyme immunoassay for determination of gluten in foods: Collaborative Study, *Journal of the Association of Official Analytical Chemists*, **74**(2), 257-264.
- Smith, P.K., Hourihane, J.O.B. and Lieberman, P., 2015, Risk multipliers for severe food anaphylaxis. *World Allergy Organization Journal* **8** (1), 30.
- Størsrud S, Yman M and Lenner R A., 2003, Gluten contamination in oat products and products naturally free from gluten, *European Food Research & Technology*, **217**(6), 481-485.
- Stein, K., 2015, Effective allergen management practices to reduce allergens in food, *In* Flanagan, S. (Ed.) *Handbook of Food Allergen Detection and Control*, Woodhead Publishing 2015, pp.103

- Spanjersberg, M.Q.I., Kruizinga, A.G., Rennen, M.A.J. and Houben, G.F., 2007, Risk assessment and food allergy: the probabilistic model applied to allergens. *Food and Chemical Toxicology* **45**(1), 49–54.
- Spanjersberg, M.Q.I., Knulst, A.C., Kruizinga, A.G., Van Duijn, G. and Houben, G.F., 2010, Concentrations of undeclared allergens in food products can reach levels that are relevant for public health. *Food Additives and Contaminants, Part A* **27**(2) 169–174.
- Stazi A V, Trecca A and Triniti B, 2008, Osteoporosis in celiac disease and in endocrine and reproductive disorders, *World Journal of Gastroenterology*, **14**(4), 498-505.
- Stubbs G. and More A., 1919, The estimation of the approximate quantity of meat in sausages and meat pastes, *Analyst*, **44**, 125-127.
- Sun H-S, Wanga F-C and Ai L-F, 2007, Determination of banned 10 azo-dyes in hot chili products by gel permeation chromatography–liquid chromatography–electrospray ionization-tandem mass spectrometry, *J Chromatog A* **1164**, 120–128
- Swan, K.E., Fitzsimons, R., Anagnostou, K., Boardman, A. and Fox, A.T., 2016, The prevention and management of anaphylaxis, *Paediatrics and Child Health* **26**, 279–286.
- Taylor, S.L., Hefle, S.L., Bindslev-Jensen, C., Bock, S.A., Burks Jr, A.W., Christie, L., Hill, D.J., Host, A., Hourihane, J.O.B., Lack, G., Metcalfe, D.D., Moneret-Vautrin, D.A., Vadas, P.A., Rance, F., Skrypec, D.J., Trautman, T.A., Yman, I.M. and Zeiger, R.S., 2002, Factors affecting the determination of threshold doses for allergenic foods: How much is too much? *Journal of Allergy and Clinical Immunology* **109**, 24–30.
- Sykes M. , Anderson D., and Parmar B., 2012, Normalisation of data from allergens proficiency tests, *Anal. Bioanal. Chem.*, **403**, 3069-3076.
- Taylor, S.L., Baumert, J.L., Kruizinga, A.G., Remington, B.C., Crevel, R.W., Brooke-Taylor, S., Allen, K.J., of Australia, T.A.B. and Houben, G., 2014, Establishment of reference doses for residues of allergenic foods: report of the VITAL expert panel. *Food and Chemical Toxicology* **63**, 9–17.
- Taylor G., 2010, Forensic enforcement : the role of the public analyst. RSC Publishing, Cambridge
- The Department of Health, Social Services and Public Safety, Food Additives Regulations (Northern Ireland) 2009, SRNI No 416 available at: http://www.opsi.gov.uk/sr/sr2009/nisr_20090416_en_1 (accessed 07 04 10)
- The Sale of Food and Drugs Act, 38 and 39 Vict. C63, 1875. 1875
- Thorburn Burns, D and Alliston G. V., 1971, Some studies on the photolytic decomposition stage in the estimation of N-nitrosamines", *J Fd. Technol.*, **6**, 433-438.
- ToxLearn 2015: U.S. National Library of Medicine, National Institutes of Health Toxicology tutorial Last Updated: January 16, 2015. Available at <https://toxlearn.nlm.nih.gov/> (accessed 30.03.2016).
- TRACE, 2016: Trace Peanut Study. Available at <http://www.tracestudy.com/> (accessed 01.07.2016).

- Turner, P.J., and Gowland, M.H. (2016) Precautionary Allergy Labelling: NO MORE TRACES! *Allergy* DOI: 10.1111/all.12961.
- Turner, A and Gorsuch, T. 1999, Report on the Review of Public Analyst Services in Northern Ireland, Department of Health & Social Services, (see para 31) available at http://archive.publicanalyst.com/N_Ireland_Report.pdf (accessed 26.07.2016)
- UK MANCP 2015: Food Standards Agency, with Food Standards Scotland, Defra, the Welsh Government, the Scottish Government and the Department of Agriculture and Rural Development, Northern Ireland, 2015, Multi-Annual National Control Plan for the United Kingdom April 2013 to March 2016 (extended and updated March 2015), https://www.food.gov.uk/sites/default/files/mancp-uk_0.pdf (accessed 07.03.2016)
- Uematsu Y, Ogimoto M, Kabashima J, Suzuki K and Ito K (2007) Fast cleanup method for the analysis of Sudan I-IV and Para Red in various foods and Paprika color (Oleoresin)...., *J. AOAC Int.*, **90**: 437-445
- Umasunthar, T., Leonardi-Bee, J., Hodes, M., Turner, P.J., Gore, C., Habibi, P., Warner, J.O. and Boyle, R.J. (2013) Incidence of fatal food anaphylaxis in people with food allergy: a systematic review and meta-analysis. *Clinical and Experimental Allergy* **43**(12), 1333–1341
- US FDA 2011: US Food and Drug Administration, Listing of Food Additive Status.
- U.S. National Library of Medicine, 2010, Medline Plus, , National Institutes of Health (2010) Phenylketonuria. Available at: <http://www.nlm.nih.gov/medlineplus/ency/article/001166.htm> (last accessed 01.08.2016)
- Ventner, C., Sommer, I., Moonesinghe, H., Grundy, J., Glasbey, G., Patil, V. and Dean, T., 2015, Health-Related Quality of Life in children with perceived and diagnosed food hypersensitivity. *Pediatric Allergy and Immunology* **26**, 126–132.
- Verhoeckx, K.C., Vissers, Y.M., Baumert, J.L., Faludi, R., Feys, M., Flanagan, S., Herouet-Guicheney, C., Holzhauser, T., Shimojo, R., van der Bolt, N. and Wichers, H., 2015, Food processing and allergenicity. *Food and Chemical Toxicology* **80**, 223–240
- Vos, E., 2000. EU food safety regulation in the aftermath of the BSE crisis. *Journal of Consumer Policy*, **23**(3), pp.227-255.
- Walker M and Stuart B, (2006), Analysis of Illegal Dyes in Chilli Powder by LC-UV, LGC/GC/2007/005, Government Chemist Programme, available at https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/469777/Analysis_of_illegal_dyes_in_chilli_powder_by_LC-UV_3nhSP.pdf (accessed 01.08.2016)
- Walker M and Stuart B (2006): Analysis of Illegal Dyes in Paprika Powder by LC-MS/MS, LGC/GC/2007/006, Government Chemist Programme, available at https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/510960/Analysis_of_illegal_dyes_in_paprika_powder_by_LC_MSMS.pdf (Accessed 01.08.2016)
- Walker, M.J., 2012, Forensic investigation of a sabotage incident in a factory manufacturing nut-free ready meals in the UK, In: J Hoorfar, Ed. *Case Studies in food safety and authenticity*, Woodhead Publishing, pp.288–295.

- Walker, M.J. and Wong, Y.C., 2014, Protection of the Agri-Food Chain by Chemical Analysis: The European Context. *In*: Rajeev Bhat, Vicente M. Gomez-Lopez (Eds) *Practical Food Safety: Contemporary Issues and Future Directions*, Wiley-Blackwell, pp 125–144.
- Walker, M.J., Burns, D.T., Elliott, C., Gowland, M.H. and Mills, E.N.C., 2016, Flawed food allergen analysis–health and supply chain risks and a proposed framework to address urgent analytical needs. *Analyst* **141**, 24–35.
- Walker 1999: Walker, M.J. “Colours and Intense sweeteners in Foods-Food Additives Survey 1999”, Department of Health Social Services and Public Safety, N.I. Environmental Health Departments/The Public Analysts Laboratory. Unpublished report.
- Walker, M.J. “Colours and Intense sweeteners in Foods-Food Additives Survey 1999”, Department of Health Social Services and Public Safety, N.I. Environmental Health Departments/The Public Analysts Laboratory. Unpublished report.
- M. J. Walker, J. McShane, D. Thorburn Burns, P. Canavan and A. Flanagan, 2009, A Cross-border Method Validation for the Determination of Intense sweeteners in Soft Drinks, J. Assoc. Publ. Analysts (JAPA Online) 37, 1 – 13 available at: http://www.apajournal.org.uk/JAPA_Vol_37_pg_1-13/japa_vol_37_pg_1-13.html
- Weber D, Cl  roux C and Godefroy S E., 2009, Emerging analytical methods to determine gluten markers in processed foods-method development in support of standard setting, *Analytical & Bioanalytical Chemistry*, **395**(1), 111-117.
- World Health Organisation monographs on selected medicinal plants, Volume 1, p 115, *Rhizoma Curcumae Longae*, WHO, Geneva, 1999
- WHO, World Health Organization (2009) Food Safety, Project to update the principles and methods for the assessment of chemicals in food, Environmental Health Criteria monograph 240.
- Wood, Roger, Foster, Lucy, Damant, Andrew, Key, Pauline.,2004, Analytical Methods for Food Additives. Woodhead Publishing. Online version available at: <http://app.knovel.com/hotlink/toc/id:kpAMFA0002/analytical-methods-food/analytical-methods-food> (accessed via RSC members' area, last accessed 01.08.2016)
- Wynter Blyth, A., 1884 (the 2nd edition). Poisons: Their Effects and Detection; a Manual for the Use Of Analytical Chemists and Experts. Charles Griffin, limited.
- Wynter Blyth, A and Wynter Blyth, M, 1909, Foods: Their Composition and Analysis, 1909 (the 4th edition), London, Griffin & Co. Ltd., pp14 – 22.
- Yoshioka N and Ichihashi K., 2008, Determination of 40 synthetic food colors in drinks and candles by high-performance liquid chromatography using a short column with photodiode array detection”, *Talanta*, 74: 1408-1413
- Zurzolo, G.A., Koplin, J.J., Ponsonby, A-L., McWilliam, V., Dharmage, S., Heine, R.G., Tang, M.L.K., Prescott, S., Campbell, D.E., Loh, R., Rueter, K., Netting, M., Frith, K., Norton, W., Said, M., Gold, M., Lee, N. A., Mathai, M., de Courten, M., and Allen, K.J. (2016) Consensus of stakeholders on precautionary allergen labelling: A report from the Centre for Food and Allergy Research. *Journal of Paediatrics and Child Health* DOI: 10.1111/jpc.13202

10 Citations of Walker's work

Citations of Walker (*et al.*'s) publications are as follows as found in 'Google Scholar' and Mendeley on 01 August 2016.

[1] A Cross-border Method Validation for the Determination of Sweeteners in Soft Drinks, **M. J. Walker**, J. McShane, D. Thorburn Burns, P. Canavan and A. Flanagan, *Journal of the Association of Public Analysts* (Online), 2009, 37, 1-14. And [2] Intense Sweeteners and Preservatives: Contemporary Regulation and Historical Baseline Data of the Nature and Amounts in Soft Drinks on open sale in Northern Ireland, **M. J. Walker**, J. Mairs and D. Thorburn Burns, *Journal of the Association of Public Analysts* (Online), 2011, 39, 1-12.

Cited by 1

Kawase, K.Y., Luchese, R.H. and Coelho, G.L., 2013. Micronized benzoic acid decreases the concentration necessary to preserve acidic beverages against *Alicyclobacillus*. *Journal of applied microbiology*, 115(2), pp.466-474.

[4] Forensically Robust Detection of the Presence of Morpholine in Apples—Proof of Principle **M. J. Walker**, K. Gray, C. Hopley, D. Bell, P. Colwell, P. Maynard and D. Thorburn Burns, *Food Analytical Methods*, 2012, 5(4), 874–880

Cited in [5] and [23].

[5] Forensically Robust Determination of the Illegal Dye Dimethyl Yellow in a Refractory Curcuma Oleoresin–Surfactant Matrix—a Case Study, **M. J. Walker**, P. Colwell, S. Biesenbruch, B. Stuart and D. Thorburn Burns, *Food Analytical Methods*, 2013, 6(2), 395–405.

Cited in [23] and a further self-citation in Gray, K.M., Walker, M.J., Burn, M.J.S., Mazur, M., Niedzwiedzka, K., Liszka, K. and Burns, D.T., 2016. Illegal Dyes in Food and Spices—A 2006 LGC LC-UV/Visible Method Reviewed and Updated for 19 Dyes. *Journal of the Association of Public Analysts* (Online), 44, pp.018-039.

[6] Food Allergy: Gambling your life on a take-away meal, I. Leitch, **M. J. Walker**, and R. Davey, *International Journal of Environmental Health Research*, 2005, 15(2), 79-87.

Cited in [9], [15] and in Walker, M.J., Burns, D.T., Elliott, C.T., Gowland, M.H. and Mills, E.C., 2016. Is food allergen analysis flawed? Health and supply chain risks and a proposed framework to address urgent analytical needs. *Analytst*, 141(1), pp.24-35. **and by 26 others as follows**

Anagnostou, K., Islam, S., King, Y., Foley, L., Pasea, L., Bond, S., Palmer, C., Deighton, J., Ewan, P. and Clark, A., 2014. Assessing the efficacy of oral immunotherapy for the desensitisation of peanut allergy in children (STOP II): a phase 2 randomised controlled trial. *The Lancet*, 383(9925), pp.1297-1304.

Bailey, S., Albardiaz, R., Frew, A.J. and Smith, H., 2011. Restaurant staff's knowledge of anaphylaxis and dietary care of people with allergies. *Clinical & Experimental Allergy*, 41(5), pp.713-717.

Gallagher, M., Worth, A., Cunningham-Burley, S. and Sheikh, A., 2012. Strategies for living with the risk of anaphylaxis in adolescence: qualitative study of young people and their parents. *Primary Care Respiratory Journal*, 21, pp.392-397.

Leftwich, J., Barnett, J., Muncer, K., Shepherd, R., Raats, M.M., Hazel Gowland, M. and Lucas, J.S., 2011. The challenges for nut-allergic consumers of eating out. *Clinical & Experimental Allergy*, 41(2), pp.243-249.

Rothstein, H., 2005. Escaping the regulatory net: Why regulatory reform can fail consumers. *Law & Policy*, 27(4), pp.520-548.

Muraro, A., Agache, I., Clark, A., Sheikh, A., Roberts, G., Akdis, C.A., Borrego, L.M., Higgs, J., Hourihane, J.B., Jorgensen, P. and Mazon, A., 2014. EAACI Food Allergy and Anaphylaxis Guidelines: managing patients with food allergy in the community. *Allergy*, 69(8), pp.1046-1057.

Pitchforth, E., Weaver, S., Willars, J., Wawrzakowicz, E., Luyt, D. and Dixon-Woods, M., 2011. A qualitative study of families of a child with a nut allergy. *Chronic illness*, p.1742395311411591.

Ansell, C., 2006. The asymmetries of governance. *What's the Beef? The Contested Governance of European Food Safety*.

Abbot, J.M., Byrd-Bredbenner, C. and Grasso, D., 2007. "Know before You Serve" Developing a Food-Allergy Fact Sheet. *Cornell Hotel and Restaurant Administration Quarterly*, 48(3), pp.274-283.

Brough, H.A., Turner, P.J., Wright, T., Fox, A.T., Taylor, S.L., Warner, J.O. and Lack, G., 2015. Dietary management of peanut and tree nut allergy: what exactly should patients avoid?. *Clinical & Experimental Allergy*, 45(5), pp.859-871.

Common, L.A., Corrigan, C.J., Smith, H., Bailey, S., Harris, S. and Holloway, J.A., 2013. How safe is your curry? Food allergy awareness of restaurant staff. *Journal of Allergy & Therapy*, 2013.

Trendelenburg, V., Enzian, N., Bellach, J., Schnadt, S., Niggemann, B. and Beyer, K., 2015. Detection of relevant amounts of cow's milk protein in non-pre-packed bakery products sold as cow's milk-free. *Allergy*, 70(5), pp.591-597.

Rothstein, H., 2009. The origins of regulatory uncertainty in the UK food safety regime. *Uncertain Risks Regulated*, pp.69-85.

Hattersley, S. and King, R., 2014. Catering e How to Keep Allergic Consumers Happy and Safe. *Risk Management for Food Allergy*, p.189.

Choi, J.H., 2012. University foodservice employees' food allergy knowledge, attitudes, practices, training and training needs.

Buttigieg, N. and Schembri, P., 2015. Towards further democratization of food consumption: Keeping the consumer informed. *Journal of Research for Consumers*, (28), p.38.

Anagnostou, K., Islam, S., King, Y., Foley, L., Pasea, L., Palmer, C., Bond, S., Ewan, P. and Clark, A., Study of induction of Tolerance to Oral Peanut: a randomised controlled trial of desensitisation using peanut oral immunotherapy in children (STOP II), EFFICACY AND MECHANISM EVALUATION, 1,(4), 2014, ISSN 2050-4365, DOI 10.3310/eme01040 National Institute for Health research

Hoogeveen, A.R., van der Fels-Klerx, H.J., Bonanno, A. and Bremer, M.G.E.G., 2016. Financial burden of allergen free food preparation in the catering business. *Quality Assurance and Safety of Crops & Foods*, 8(2), pp.301-308.

Lee, Y.M. and Kwon, J., 2011. Exploration of Attitudes and Behaviors of Consumer with Food Allergies about Dining Out: A Focus Group Study.

Cohen, S.J., 2010. Illness Uncertainty, Ways of Coping, and Psychological Adjustment Among 18--25-Year-Olds with Anaphylactic Food Allergy.

WOJTYNIAK, K., KRAUZE, A. and SZCZĘSNA, M., 2013. Znajomość zagadnień alergii pokarmowej wśród pracowników restauracji.

Konturek, P.C., Zopf, Y., Hahn, E.G. and Raithel, M., 2007. Ernährung und Nahrungsmittelallergie. *Ernährung & Medizin*, 22(03), pp.127-132.

Konturek, P.C., Zopf, Y., Hahn, E.G. and Raithel, M., 2007. Ernährung und Nahrungsmittelallergie-Allergische Mechanismen exakt diagnostizieren. *Notfall & Hausarztmedizin*, 33(11), pp.512-517.

[8] Food Allergen Detection: A Literature Review 2004 – 2007, **M. J. Walker**, P. Colwell, S. Elahi, K. Gray and I. Lumley, *Journal of the Association of Public Analysts* (Online), 2008, 36, 1-18.

Cited in [13], and in Walker, M.J., Burns, D.T., Elliott, C.T., Gowland, M.H. and Mills, E.C., 2016. Is food allergen analysis flawed? Health and supply chain risks and a proposed framework to address urgent analytical needs. *Analyst*, 141(1), pp.24-35 **and by 4 others:**

Surojanametakul, V., Doi, H., Shibata, H., Mizumura, T., Takahashi, T., Varayanond, W., Wannapinpong, S., Shoji, M., Ito, T. and Tamura, H., 2011. Reliable enzyme-linked immunosorbent assay for the determination of coconut milk proteins in processed foods. *Journal of agricultural and food chemistry*, 59(6), pp.2131-2136.

Korte, R., Lepski, S. and Brockmeyer, J., 2016. Comprehensive peptide marker identification for the detection of multiple nut allergens using a non-targeted LC–HRMS multi-method. *Analytical and bioanalytical chemistry*, 408(12), pp.3059-3069.

Petrášová, M., Pospiech, M., Tremlová, B. and Randulová, Z., 2015. Immunofluorescence detection of milk protein in meat products. *Potravinárstvo*, 9(1), pp.101-105.

BRYCHTA, J., HONZLOVÁ, A., BULAWOVÁ, H. and ŠIMEK, B., Výsledky kontroly přítomnosti alergenů v masných výrobcích.

[9] Awareness of coeliac disease and the gluten status of 'gluten-free' food obtained on request in catering outlets in Ireland, J. McIntosh, A. Flanagan, N. Madden, M. Mulcahy, L. Dargan, **M. J. Walker** & D. Thorburn Burns, *International Journal of Food Science & Technology*, 2011, 46, 1569-1574.

Cited in Walker, M.J., Burns, D.T., Elliott, C.T., Gowland, M.H. and Mills, E.C., 2016. Is food allergen analysis flawed? Health and supply chain risks and a proposed framework to address urgent analytical needs. *Analyst*, 141(1), pp.24-35, **and by 6 others:**

Aziz, I., Karajeh, M.A., Zilkha, J., Tubman, E., Fowles, C. and Sanders, D.S., 2014. Change in awareness of gluten-related disorders among chefs and the general public in the UK: a 10-year follow-up study. *European journal of gastroenterology & hepatology*, 26(11), pp.1228-1233.

Hattersley, S. and King, R., 2014. Catering e How to Keep Allergic Consumers Happy and Safe. *Risk Management for Food Allergy*, p.189.

Silvester, J.A., Graff, L.A., Rigaux, L., Walker, J.R. and Duerksen, D.R., 2016. Symptomatic suspected gluten exposure is common among patients with coeliac disease on a gluten-free diet. *Alimentary Pharmacology & Therapeutics*.

Bentley, A.C., 2013. *The Development of Gluten-Free Milk-Free French Bread* (Doctoral dissertation, New Jersey Medical School).

Sálková, D. and Hosková, P., 2016. Consumer behavior-people intolerant to gluten and gluten-free offer in the public catering sector. *Emirates Journal of Food and Agriculture*, 28(4), p.271.

ŠÁLKOVÁ, D. and HES, A., 2015. Gluten-Free Food—the Influence of Selected Qualitative Characteristics on Consumer Decision Making of Coeliacs in Hospitality Establishments. *Czech Journal of Food Sciences*, 33(6), pp.513-517.

[10] Food allergy, a summary of recent cases in the criminal and civil courts of the UK, Oral presentation, H. M. Gowland and **M. J. Walker**, 2011, *Clinical and Translational Allergy*, Volume 1, Supplement 1 (2011), O1.

Cited in [13] and by Gowland, H., 2013. Reactions, Regulation and Risk: protecting consumers with food allergies and intolerances. *Perspectives in public health*, 133(6), pp.306-307.

[11] A Mass Spectrometry-based Reference Method for the Analysis of Lysozyme in Wine and the Production of Certified Reference Materials, A. Cryar, C. Pritchard, W. Burkitt, **M. J. Walker**, G. O'Connor and M. Quaglia, *Journal of the Association of Public Analysts* (Online), 2012, 40, 77-80.

Cited by 3:

Prado, M., Ortea, I., Vial, S., Rivas, J., Calo-Mata, P. and Barros-Velázquez, J., 2015. Advanced DNA-and protein-based methods for the detection and investigation of food allergens. *Critical reviews in food science and nutrition*, (just-accepted), pp.00-00.

Yang, W., Liqing, W., Fei, D., Bin, Y., Yi, Y. and Jing, W., 2014. Development of an SI-Traceable HPLC–Isotope Dilution Mass Spectrometry Method To Quantify β -Lactoglobulin in Milk Powders. *Journal of agricultural and food chemistry*, 62(14), pp.3073-3080.

Ortea, I., O'Connor, G. and Maquet, A., 2016. Review on proteomics for food authentication. *Journal of Proteomics*.

[12] Forensic investigation of a sabotage incident in a factory manufacturing nut-free ready meals in the UK, **M. J. Walker**, *In: J Hoorfar, Ed. Case Studies in food safety and authenticity*, Woodhead Publishing, 2012, pp 288-295

Cited in [15] and in Walker, M.J., Burns, D.T., Elliott, C.T., Gowland, M.H. and Mills, E.C., 2016. Is food allergen analysis flawed? Health and supply chain risks and a proposed framework to address urgent analytical needs. *Analyst*, 141(1), pp.24-35.

[13] Towards Absolute Quantification of Allergenic Proteins in Food—Lysozyme in Wine as a Model System for Metrologically Traceable Mass Spectrometric Methods and

- Certified Reference Materials, A. Cryar, C. Pritchard, W. Burkitt, **M. J. Walker**, G. O'Connor, D. Thorburn Burns and M. Quaglia, *Journal of the Association of Analytical Communities International*, 2013, 96, 1350-1361
- Cited in Walker, M.J., Burns, D.T., Elliott, C.T., Gowland, M.H. and Mills, E.C., 2016. Is food allergen analysis flawed? Health and supply chain risks and a proposed framework to address urgent analytical needs. *Analyst*, 141(1), pp.24-35 **and by 6 others**:
- Monaci, L., Pilolli, R., De Angelis, E., Godula, M. and Visconti, A., 2014. Multi-allergen detection in food by micro high-performance liquid chromatography coupled to a dual cell linear ion trap mass spectrometry. *Journal of Chromatography A*, 1358, pp.136-144.
- Pilolli, R., De Angelis, E., Godula, M., Visconti, A. and Monaci, L., 2014. Orbitrap™ monostage MS versus hybrid linear ion trap MS: application to multi-allergen screening in wine. *Journal of Mass Spectrometry*, 49(12), pp.1254-1263.
- Di Girolamo, F., Muraca, M., Mazzina, O., Lante, I. and Dahdah, L., 2015. Proteomic applications in food allergy: food allergenomics. *Current opinion in allergy and clinical immunology*, 15(3), pp.259-266.
- Jiang, Y., Yang, H., Quan, S., Liu, Y., Shen, P. and Yang, L., 2015. Development of certified matrix-based reference material of genetically modified rice event TT51-1 for real-time PCR quantification. *Analytical and bioanalytical chemistry*, 407(22), pp.6731-6739.
- Peñas, E., di Lorenzo, C., Uberti, F. and Restani, P., 2015. Allergenic Proteins in Enology: A Review on Technological Applications and Safety Aspects. *Molecules*, 20(7), pp.13144-13164.
- Monaci, L., Pilolli, R., De Angelis, E., Carone, R. and Pascale, M., 2016. LC-tandem mass spectrometry as a screening tool for multiple detection of allergenic ingredients in complex foods. *ACTA IMEKO*, 5(1), pp.5-9.

[14] A multi-laboratory evaluation of a clinically-validated incurred quality control material for analysis of allergens in food, P. E. Johnson, N. M. Rigby, J. R. Dainty, A. R. Mackie, U. U. Immer, A. Rogers, P. Titchener, M. Shoji, A. Ryan, L. Mata, H. Brown, T. Holzhauser, V. Dumont, J. A. Wykes, **M. J. Walker**, J. Griffin, J. White, G. Taylor G., B. Popping, R. Crevel, S. Miguel, P. Lutter, F. Gaskin, T. B. Koerner, D. Clarke, R. Sherlock, A. Flanagan, C.H. Chan, E.N. Clare Mills, *Food Chemistry*, 2014, 148, 30-36.

Cited in Walker, M.J., Burns, D.T., Elliott, C.T., Gowland, M.H. and Mills, E.C., 2016. Is food allergen analysis flawed? Health and supply chain risks and a proposed framework to address urgent analytical needs. *Analyst*, 141(1), pp.24-35 **and by 10 others**:

- Muraro, A., Hoffmann-Sommergruber, K., Holzhauser, T., Poulsen, L.K., Gowland, M.H., Akdis, C.A., Mills, E.N.C., Papadopoulos, N., Roberts, G., Schnadt, S. and Ree, R., 2014. EAACI Food Allergy and Anaphylaxis Guidelines. Protecting consumers with food allergies: understanding food consumption, meeting regulations and identifying unmet needs. *Allergy*, 69(11), pp.1464-1472.
- Koeberl, M., Clarke, D. and Lopata, A.L., 2014. Next generation of food allergen quantification using mass spectrometric systems. *Journal of proteome research*, 13(8), pp.3499-3509.
- DunnGalvin, A., Chan, C.H., Crevel, R., Grimshaw, K., Poms, R., Schnadt, S., Taylor, S.L., Turner, P., Allen, K.J., Austin, M. and Baka, A., 2015. Precautionary

allergen labelling: perspectives from key stakeholder groups. *Allergy*, 70(9), pp.1039-1051.

Dang, T.D., Mills, C.E. and Allen, K.J., 2014. Determination of the clinical egg allergy phenotypes using component-resolved diagnostics. *Pediatric Allergy and Immunology*, 25(7), pp.639-643.

Reese, I., Holzhauser, T., Schnadt, S., Dölle, S., Kleine-Tebbe, J., Raithel, M., Worm, M., Zuberbier, T. and Vieths, S., 2015. Allergen and allergy risk assessment, allergen management, and gaps in the European Food Information Regulation (FIR). *Allergo Journal International*, 24(6), pp.180-184.

Kapila, R. and Kapila, S., 2015. Analytical Tools for Assessment of Allergens in Foods. *Advanced Techniques and Novel Approaches for Quality and Safety Evaluation of Dairy Foods*, p.155.

Muraro, A., Hoffmann-Sommergruber, K., Holzhauser, T., Poulsen, L.K., Gowland, M.H., Akdis, C.A., Mills, E.N.C., Papadopoulos, N., Roberts¹¹, G., Schnadt¹⁴, S. and van Ree¹⁵, R., PROTECTING CONSUMERS WITH FOOD ALLERGIES. *Food Allergy and Anaphylaxis Guidelines*, p.259.

Reese, I., Holzhauser, T., Schnadt, S., Dölle, S., Kleine-Tebbe, J., Raithel, M., Worm, M., Zuberbier, T. and Vieths, S., 2015. Allergen-und Allergierisikobewertung, Allergenmanagement und Lücken der europäischen Lebensmittelinformationsverordnung (LMIV). *Allergo Journal*, 24(6), pp.28-32.

Hirst, B. and Miguel, S.J., 2014. Validation, standardisation and harmonisation of analytical methods and test kits for detecting allergens in food. *Handbook of Food Allergen Detection and Control*, p.295.

Masiá Reyes, A. and Picó García, Y., Quality Control in Foods, State of the Art and Prospects. *Encyclopedia of Analytical Chemistry*.

[15] Food Allergy, a summary of 8 cases in the UK criminal and civil courts: effective last resort for vulnerable consumers?, M. H. Gowland and **M.J. Walker**, 2014, *Journal of the Science of Food and Agriculture*, 2015, 95(10), 1979-1990.

Cited by 1:

Zurzolo, G.A., de Courten, M., Koplin, J., Mathai, M.L. and Allen, K.J., 2016. Is advising food allergic patients to avoid food with precautionary allergen labelling out of date?. *Current opinion in allergy and clinical immunology*, 16(3), pp.272-277.

[17] Nitrogen Factors for Atlantic Salmon, *Salmo salar*, farmed in Scotland and in Norway and for the derived ingredient, "Salmon Frame Mince", in Fish Products. P. Colwell, S. L. R. Ellison, **M. J. Walker**, S. Elahi, D. Thorburn Burns and K. Gray, *Journal of the Association of Public Analysts* (Online), 2011, 39, 44-78.

Cited in [16] and by 2 others

van Ruth, S.M., Brouwer, E., Koot, A. and Wijten, M., 2014. Seafood and Water Management. *Foods*, 3(4), pp.622-631.

62, A.M.C.A.N., 2014. Seafood nitrogen factors. *Analytical Methods*, 6(13), pp.4490-4492.

[18] Can Analytical Chemists do Molecular Biology? A Survey of the Up skilling of the UK Official Food Control System in DNA Food Authenticity Techniques, M. Woolfe, T. Gurung and **M. J. Walker**, *Journal of Food Control*, 2013, **33**, 385-392.

Cited in [20] and by 6 others:

Griffiths, A.M., Sotelo, C.G., Mendes, R., Pérez-Martín, R.I., Schröder, U., Shorten, M., Silva, H.A., Verrez-Bagnis, V. and Mariani, S., 2014. Current methods for seafood authenticity testing in Europe: is there a need for harmonisation?. *Food Control*, **45**, pp.95-100.

Khaksar, R., Carlson, T., Schaffner, D.W., Ghorashi, M., Best, D., Jandhyala, S., Traverso, J. and Amini, S., 2015. Unmasking seafood mislabeling in US markets: DNA barcoding as a unique technology for food authentication and quality control. *Food Control*, **56**, pp.71-76.

Porcari, A.M., Fernandes, G.D., Barrera-Arellano, D., Eberlin, M.N. and Alberici, R.M., 2016. Food quality and authenticity screening via easy ambient sonic-spray ionization mass spectrometry. *Analyst*, **141**(4), pp.1172-1184.

Mueller, S., Handy, S.M., Deeds, J.R., George, G.O., Broadhead, W.J., Pugh, S.E. and Garrett, S.D., 2015. Development of a COX1 based PCR-RFLP method for fish species identification. *Food Control*, **55**, pp.39-42.

Wen, J. and Zeng, L., 2014. Use of species-specific PCR for the identification of 10 sea cucumber species. *Chinese Journal of Oceanology and Limnology*, **32**, pp.1257-1263.

李溪盛 and 马莺, 2014. DNA 指纹技术在食品掺假鉴定中的应用. *中国甜菜糖业*, (004), pp.44-50.

[20] Horse meat in beef products, species substitution, **M. J. Walker**, M. Burns and D. Thorburn Burns, *Journal of the Association of Public Analysts* (Online), 2013, **41**, 67-106.

Cited in [21], and in Walker, M.J., Burns, D.T., Elliott, C.T., Gowland, M.H. and Mills, E.C., 2016. Is food allergen analysis flawed? Health and supply chain risks and a proposed framework to address urgent analytical needs. *Analyst*, **141**(1), pp.24-35, **and by 8 others:**

Di Giuseppe, A.M., Giarretta, N., Lippert, M., Severino, V. and Di Maro, A., 2015. An improved UPLC method for the detection of undeclared horse meat addition by using myoglobin as molecular marker. *Food chemistry*, **169**, pp.241-245.

Burns, D.T., The Analytical Division's contributions to accounts of the history of Analytical Chemistry in the UK, Ireland and mainland Europe.

Kýrová, V., Ostrý, V., Surmanová, P., Procházková, I., Řehůřková, I., Ruprich, J. and Jechová, M., Horse meat in beef products and adulteration of gadoid fish meat products in the Czech Republic.

Zahran, D. and Hagag, S., 2015. Use of molecular biology techniques in the detection of fraud meat in the Egyptian market. *African Journal of Biotechnology*, **14**(5), pp.360-364.

Ugochukwu, A.I., Hobbs, J.E., Phillips, P.W. and Gray, R., 2015. An economic analysis of private incentives to adopt DNA barcoding technology for fish species authentication in Canada 1. *Genome*, **58**(12), pp.559-567.

Busby, E. and Burns, M., 2014. Method Verification of the LOD Associated with PCR Approaches for the Detection of Horse Meat. *Journal of the Association of Public Analysts (Online)*, 42, pp.001-017.

Burns, D.T., Walker, M.J., Buchberger, W. and Worsfold, P., 2016. European Analytical Column No. 44. *J. Serb. Chem. Soc.*, 81(4), pp.469-474.

Cawthorn, D.M. and Hoffman, L.C., 2016. Controversial cuisine: A global account of the demand, supply and acceptance of “unconventional” and “exotic” meats. *Meat science*.

[21] The adulteration of food, lessons from the past, with reference to butter, margarine and fraud, European Food Research and Technology, H. Deelstra, D.Thorburn Burns and **M. J. Walker**, *European Food Research and Technology*, 2014, 239, 725-744.

Cited by 6:

Tomaszewska-Gras, J., DSC coupled with PCA as a tool for butter authenticity assessment. *Journal of Thermal Analysis and Calorimetry*, pp.1-8.

Nedeljković, A., Rösch, P., Popp, J., Miočinović, J., Radovanović, M. and Pudja, P., 2016. Raman Spectroscopy as a Rapid Tool for Quantitative Analysis of Butter Adulterated with Margarine. *Food Analytical Methods*, 9(5), pp.1315-1320.

Xiaobo, Z., Xiaowei, H. and Povey, M., 2016. Non-invasive sensing for food reassurance. *Analyst*, 141(5), pp.1587-1610.

Burns, D.T., Walker, M.J., Buchberger, W. and Worsfold, P., 2016. European Analytical Column No. 44. *J. Serb. Chem. Soc.*, 81(4), pp.469-474.

George, P.C., 2015. The evolution of food consumption in Romania with impact on human health. *Universitatii Bucuresti. Analele. Seria Stiinte Economice si Administrative*, 9, p.45.

Louise, M., 2016. Food fraud: policy and food chain. *Current Opinion in Food Science*.

[22] Referee analysis of suspected irradiated food, S. Elahi, I. Straub, K. Thurlow, P. Farnell, and **M. J. Walker**, *Journal of Food Control*, 2008, 19, 269-277.

Cited by 12

Akram, K., Ahn, J.J. and Kwon, J.H., 2012. Analytical methods for the identification of irradiated foods. *Ionizing radiation: Applications, sources and biological effects*, pp.1-36.

Kim, B.K., Akram, K., Kim, C.T., Kang, N.R., Lee, J.W., Ryang, J.H. and Kwon, J.H., 2012. Identification of low amount of irradiated spices (red pepper, garlic, ginger powder) with luminescence analysis. *Radiation Physics and Chemistry*, 81(8), pp.1220-1223.

Ahn, J.J., Akram, K., Baek, J.Y., Kim, Y.J., Kim, M.Y., Jeong, I.Y. and Kwon, J.H., 2012. Inter-laboratory study to define the temperature interval for a thermoluminescence heating unit used to identify irradiated food. *Food Science and Biotechnology*, 21(3), pp.853-857.

Ahn, J.J., Akram, K., Shahbaz, H.M. and Kwon, J.H., 2013. Effectiveness of luminescence analysis to identify gamma-irradiated shrimps: Effects of grinding, mixing and different methods of mineral separation. *Food research international*, 54(1), pp.416-422.

张立雯, 江英桥, 林彤, 毕福钧 and 于永洲, 2011.
中药辐照检测研究现状概述. *中药材*, 34(3), pp.482-487.

周虹, 毛建霏, 罗玲 and 雷绍荣, 2011.
光释光法快速筛查辐照方便食品香辛料及脱水蔬菜. *西南农业学报*, 24(6), pp.2355-2358.

Akram, K., Ahn, J.J., Shahbaz, H.M., Jo, D. and Kwon, J.H., 2013. Effectiveness of thermoluminescence analysis to detect low quantity of gamma-irradiated component in non-irradiated mushroom powders. *Journal of Luminescence*, 136, pp.395-400.

Park, E.R., Kang, H.S., Ahn, H.J., An, K.A., Cho, S.Y., Kim, H.Y., Kim, D.S., Kim, D.H. and Kang, C.S., 2010. Analysis of the Detection Characteristics of Irradiated Dried Spices and Herbs by Photostimulated Luminescence (PSL). *Korean Journal of Food Science and Technology*, 42(2), pp.136-141.

Jo, Y., Kyung, H.K., Park, H.J. and Kwon, J.H., 2016. Irradiated fruits can be identified by detecting radiation-induced markers with luminescence and ESR analyses for different trading fruits. *Applied Biological Chemistry*, 59(1), pp.59-65.

Bai, Y., Jiang, W., Lin, H., Liu, Z. and Liu, Z., 2014. Identification of gamma-irradiated Chinese herbs by thermoluminescence analysis. *Journal of Radioanalytical and Nuclear Chemistry*, 301(1), pp.125-131.

[23] *Quis custodiet* – a Review of the Resolution of Disputed Chemical Results in the UK Official Feed and Food Control System 2010 – 2011, **M. J. Walker** and K. Gray, *Journal of the Association of Public Analysts* (Online) 2013, 41, 1–27.

Cited in [20], [25], Points, J., Burns, D.T. and Walker, M.J., 2015. Forensic issues in the analysis of trace nitrofurans veterinary residues in food of animal origin. *Food Control*, 50, pp.92-103., **and** Walker, M.J., Burns, D.T., Elliott, C.T., Gowland, M.H. and Mills, E.C., 2016. Is food allergen analysis flawed? Health and supply chain risks and a proposed framework to address urgent analytical needs. *Analyst*, 141(1), pp.24-35, **and by 1 other:**

Pearson, R.A., Evans, C. and Bendall, J.G., 2016. Nitrofurazone quantification in milk at the European Union minimum required performance limit of 1 ng g⁻¹: circumventing the semicarbazide problem. *Food Additives & Contaminants: Part A*, pp.1-13.

11 Appendix 1 Author Contributions

Volume 2 containing the publications has not been digitised due to copyright restrictions.